



Aalborg Universitet

AALBORG UNIVERSITY  
DENMARK

## Experimental knee-related pain enhances attentional interference on postural control

Suda, Eneida Yuri; Hirata, Rogerio Pessoto; Palsson, Thorvaldur; Vuillerme, Nicolas; Sacco, Isabel C N; Graven-Nielsen, Thomas

*Published in:*  
European Journal of Applied Physiology

*DOI (link to publication from Publisher):*  
[10.1007/s00421-019-04192-9](https://doi.org/10.1007/s00421-019-04192-9)

*Publication date:*  
2019

*Document Version*  
Accepted author manuscript, peer reviewed version

[Link to publication from Aalborg University](#)

*Citation for published version (APA):*  
Suda, E. Y., Hirata, R. P., Palsson, T., Vuillerme, N., Sacco, I. C. N., & Graven-Nielsen, T. (2019). Experimental knee-related pain enhances attentional interference on postural control. *European Journal of Applied Physiology*, 119(9), 2053-2064. <https://doi.org/10.1007/s00421-019-04192-9>

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

### Take down policy

If you believe that this document breaches copyright please contact us at [vbn@aub.aau.dk](mailto:vbn@aub.aau.dk) providing details, and we will remove access to the work immediately and investigate your claim.

Click here to view linked References

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

EXPERIMENTAL KNEE-RELATED PAIN ENHANCES ATTENTIONAL INTERFERENCE ON POSTURAL CONTROL

Eneida Yuri Suda<sup>1</sup>, Rogerio Pessoto Hirata<sup>2\*</sup>, Thorvaldur Palsson<sup>2</sup>, Nicolas Vuillerme<sup>3</sup>, Isabel C N Sacco<sup>1</sup>,  
Thomas Graven-Nielsen<sup>4</sup>

Laboratory of Biomechanics of Human Movement, Dept. Physical Therapy, Speech and Occupational  
Therapy, School of Medicine, University of Sao Paulo, Sao Paulo, Brazil

Department of Health Science and Technology, SMI, Faculty of Medicine, Aalborg University, Aalborg,  
Denmark

Univ. Grenoble-Alpes, EA AGEIS, Grenoble, France & Institut Universitaire de France

Center for Neuroplasticity and Pain (CNAP), SMI, Department of Health Science and Technology, Aalborg  
University, Denmark

Original article for: European Journal of Applied Physiology

Number of Pages: 24

Number of words: 500983

Number of figures: 4

Number of tables: 3

Abstract: 249

\*Corresponding author:

Associate Professor Rogerio Pessoto Hirata, Ph.D.

Center for Sensory Motor Interaction, SMI, Department of Health Science and Technology, Aalborg University,  
Aalborg, Denmark

Department of Health Science and Technology

Aalborg University, Fredrik Bajersvej 7D-3

9220 Aalborg E, Denmark

E-mail: [rirata@hst.aau.dk](mailto:rirata@hst.aau.dk)

Acknowledgement

Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation  
(DNRF121). The authors thank the State of São Paulo Research Foundation (FAPESP) for the Suda scholarship  
(FAPESP 2017/15449-4, 2015/00214-6).

Formatted: No underline, Font color: Auto

Field Code Changed

## Abstract

**Purpose:** To quantify how postural stability is modified during experimental pain while performing different cognitively demanding tasks.

**Methods:** Sixteen healthy young adults participated in the experiment. Pain was induced by intramuscular injection of hypertonic saline solution (1mL, 6%) in both vastus medialis and vastus lateralis muscles (0.9% isotonic saline was used as control). The participants stood barefoot in tandem position for one minute on a force plate. Center of pressure (CoP) was recorded before and immediately after injections, while performing two cognitive tasks: (i) counting forwards by adding one; (ii) counting backwards by subtracting three. CoP variables – total area of displacement, velocity in anterior-posterior (AP-velocity) and medial-lateral (ML-velocity) directions, and CoP sample entropy in anterior-posterior and medial-lateral directions were displayed as the difference between the values obtained after and before each injection and compared between tasks and injections.

**Results:** CoP total area ( $-84.5 \pm 145.5$  vs.  $28.9 \pm 78.5$  cm<sup>2</sup>) and ML-velocity ( $-1.71 \pm 2.61$  vs.  $0.98 \pm 1.93$  cm/s) decreased after the painful injection vs. Control injection while counting forward ( $P < 0.05$ ). CoP total area ( $112.8 \pm 53.9$  vs.  $-84.5 \pm 145.5$  cm<sup>2</sup>), ML-velocity ( $-0.34 \pm 1.92$  vs.  $-1.71 \pm 2.61$  cm/s) and AP-velocity ( $1.07 \pm 2.35$  vs.  $-0.39 \pm 1.82$  cm/s) increased while counting backwards vs. forwards after the painful injection ( $P < 0.05$ ).

**Conclusion:** Pain interfered with postural stability according to the type of cognitive task performed, suggesting that pain may occupy cognitive resources, potentially resulting in poorer balance performance.

**Keywords:** postural stability, center of pressure, attention, distraction, pain

1		
2		
3		
4		
5		
6		
7		
53	8	<b>List of abbreviations</b>
54	9	
55	10	
	11	ANOVA      Analysis of variance
56	12	
	13	au      Arbitrary units
57	14	
	15	CoP      Center of pressure
58	16	
	17	SaEn      Sample entropy
59	18	
	19	SD      Standard deviation
60	20	
	21	VAS      Visual analogue scale
61	22	
	23	VM      Vastus medialis
62	24	
	25	VL      Vastus lateralis
63	26	
	27	
	28	
	29	
	30	
	31	
	32	
	33	
	34	
	35	
	36	
	37	
	38	
	39	
	40	
	41	
	42	
	43	
	44	
	45	
	46	
	47	
	48	
	49	
	50	
	51	
	52	
	53	
	54	
	55	
	56	
	57	
	58	
	59	
	60	
	61	
	62	
	63	
	64	
	65	

## 1. Introduction

Controlling of upright posture requires a significant amount of attention to ~~constantly~~ gather information from the body and the environment and to generate adapted and accurate muscle activation for postural control (Morasso and Sanguineti 2002). Although the majority of postural control is regulated via automatic neural processes (Bronstein and Buckwell 1997), higher cortical centers are significantly involved in processing sensory information to plan and execute the best motor strategy for postural control (Winter 1995). In daily life, postural control is challenging as several tasks simultaneously compete for the cognitive resources available (Woollacott and Shumway-Cook 2002), limited by the capacity of higher centers to process sensory information (Kahneman 1973). Therefore, sharing attentional resources may cause impairments in the performance of daily living activities (Brauer et al. 2004). ~~Evidence suggests that~~ For example, competition for cognitive resources during tasks involving postural stability results in body stability being prioritized over secondary tasks (Liston et al. 2014).

Dual tasks paradigms, where subjects perform an additional task during ~~quiet~~ standing, are employed to quantify the extent to which attention is associated with postural control. Decreases in postural sway while performing a secondary task compared with control conditions have been reported (Andersson et al. 2002; Pellecchia 2003) whereby focusing the attention on standing as still as possible increased postural sway compared with conditions without similar instructions (Vuillerme and Nafati 2007). Altogether, these results suggest that postural control demands attention (Woollacott and Shumway-Cook 2002) and that simultaneous cognitive loading plays an important role in balance stability (Swan et al. 2007).

Although detrimental effects of cognitive loading on postural sway during unperturbed standing are more commonly reported for older adults and patients, studies using dual-task approaches in young ~~and~~ ~~control~~ subjects show controversial results (Huxhold et al. 2006; Fraizer and Mitra 2008). Young healthy subjects have probably more ability to allocate the attentional resources ~~during upright standing~~ without

sacrificing postural stability, showing that a system without impairments prioritizes postural stability when dealing with dual-cognitive tasks (Siu and Woollacott 2007).

Evidence suggests that Subjects with pain demonstrate increased postural sway compared with controls (Hirata et al. 2011). ~~Among several~~ A potential possible explanations for this finding, ~~one hypothesis~~ is that the increased postural sway may relate to a disrupting effect of nociceptive stimuli on attention to other simultaneous non-nociceptive tasks (Eccleston et al. 1999), underlining that processing of nociceptive stimuli is cognitively demanding (Veldhuijzen et al. 2006). Thus, the execution of cognitive tasks during pain might interfere with postural control. Although previous studies have shown that patients with pain present impaired balance while performing a secondary cognitive task in comparison to health subjects (Van Daele et al. 2010; Larivière et al. 2013; Mazaheri et al. 2014; Sherafat et al. 2014; Etemadi et al. 2016; Levinger et al. 2016), it is not clear yet the isolate effect of pain ~~in these conditions and comparisons, since in clinical pain populations, besides pain, other factors like~~ reduced muscle strength, reduced flexibility and degenerative changes at the affected segment also cause both stiffness and instability in patients suffering from chronic pain (Knoop et al. 2012). Therefore, further investigation of the interaction between pain, cognition and postural stability is warranted. This investigation is of particular interest for clinical practice since there are evidences that attention can be directed away from pain using some specific strategies (Van Ryckeghem et al. 2018). If selective attention could be directed away from the painful stimulus and modify the deleterious effect of muscle pain on postural control, these results could have important implications for clinical settings. Likewise, if the execution of cognitive tasks impairs postural control in the presence of pain, this should also be taken into account in rehabilitation context.

Considering that posture can be defined as the dynamic stability of a continuous moving body (Harbourne and Stergiou 2003; Madeleine et al. 2011), nonlinear analysis of the dynamic structure of the center of pressure (CoP) time series would contribute to understand the physiological complexity of posture by accessing motor patterns that would be implicit in the CoP variability. Sample entropy (SaEn) measures

variations in the system output along time, ~~which is independent of the signal magnitude (Slifkin and Newell 1999; Richman and Moorman 2000).~~ Therefore, measures of physiological complexity of the postural sway during quiet standing may relate to the system functionality as they are defined as the capacity of generating adaptive answers to an ever-changing environment such as controlling posture (Manor et al. 2010). SaEn provides a measure of “orderly structure” within the time series since it tests if there are any repeated patterns of various lengths, including the ones that are not repeated at regular intervals (Duarte and Sternad 2008). So, the lower the SaEn values are, the higher the similarity and lesser the complexity in the temporal series is (Richman and Moorman 2000). ~~SaEn has been used to measure the structure of the CoP variability (Roerdink et al. 2006; Donker et al. 2007; Duarte and Sternad 2008; Stins et al. 2009) and thus address the complexity of the signal.~~

~~Most definitions of complexity are driven by operational considerations on the number of system elements and their functional interactions. Therefore, c~~Complexity depends on the number of structural components of the system, the existing coupling among these components and how this interaction is influenced by the intrinsic dynamic properties of the system and the motor task demands (Vaillancourt and Newell 2002). Thus, if the presence of pain and the execution of a cognitive task are both concurring with the attentional resources used in postural control, then the coupling between the components of the system responsible for balance may be affected and, consequently, the complexity of the postural sway is affected.

~~The literature shows that the e~~Execution of a concurrent cognitive task during standing increases the complexity of the postural sway, and this increase has been attributed to a more automatized postural sway, when less attention is directed to the balance control (Donker et al. 2007; Stins et al. 2009; Kuczyński et al. 2011). On the other hand, there is some evidence that the complexity of postural control decreases with pain. ~~Søndergaard et al. (2010) found a decrease SaEn of CoP displacement~~ during sitting with increased perceived discomfort in healthy young subjects (Søndergaard et al. 2010). ~~The same~~Similar finding was reported in young subjects with transient acute episode of low back pain during two continuous hours of

standing, but without history of low back pain (Fewster et al. 2017), showing a relation between the occurrence of pain and the decrease in CoP complexity. Therefore, examining the complexity of postural sway in a dual task context and the effect of experimental pain in this condition may improve the understanding of the decrease in postural stability (Levinger et al. 2016) and complexity (Fewster et al. 2017) that may exist as a result of pain in an otherwise healthy system.

The aim of this study was to quantify how postural stability, ~~i.e., CoP sway~~ [(CoP sway velocity and area of displacement) and ~~CoP~~ complexity (CoP SaEn)], is modified during experimental pain while performing a cognitive task. It was hypothesized that (i) the kind of cognitive task (more or less demanding) in a non-painful condition will not interfere with CoP sway or CoP complexity, since the system would have enough cognitive resources to overcome it; (ii) experimental pain will increase CoP sway and decrease CoP complexity, regardless the type of cognitive task performed; (iii) the presence of experimental pain while performing a difficult cognitive task will overload the cognitive resources and impair postural stability, increasing CoP sway and decreasing CoP complexity.

## 2. Methods

### 2.1. Subjects

Sixteen young adults, all university students, (to control for the effect of education level on multitasking performance (Voos et al. 2015)), participated in the experiment – 8 males (mean  $\pm$  SD: age =  $26.9 \pm 2.8$  years; body mass =  $74.9 \pm 13.8$  kg; height =  $1.76 \pm 0.08$  m) and 8 females (mean  $\pm$  SD: age =  $27.1 \pm 4.0$  years; body mass =  $68.8 \pm 5.2$  kg; height =  $1.68 \pm 0.06$  m). The exclusion criteria were body mass index above  $25 \text{ kg/m}^2$ , pregnancy, drug addiction, previous neurologic, musculoskeletal or mental illness, lack of ability to cooperate, current use of medications (e.g. analgesics, anti-inflammatory medicine), consumption of alcohol, caffeine, nicotine or painkillers 8 hours prior to the data collection, recent history of acute pain affecting the ~~upper-lower~~ limb and/or trunk, past history of chronic pain conditions, participation in other pain trials throughout the study period. All procedures performed in studies involving human participants



were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the local Ethics Committee (N-20120077). This sample size was calculated to detect a minimum difference of 40% in the CoP area assuming type error 1 as 5% and power of 80% between the conditions before and after the induction of experimental pain. All participants gave signed informed consents prior to inclusion in the study.

## 2.2. Experimental protocol

Since in healthy individuals approximately 70% of the information used for controlling posture originates from proprioceptive systems (Peterka 2003), we controlled the effect of different footwear on postural control by asking the subjects to stand barefoot during the experiment. The participants stood on a triangular force plate that measures vertical forces (Good Balance System, Metitur, Jyväskylä, Finland; dimensions: equilateral triangle – 800-mm; sampling frequency: 50-Hz as suggested by the International Society for Posture and Gait Research Standardization Committee (Scoppa et al. 2013)). This is a valid and reliable system for postural sway measurements (Era et al. 2006; Ha et al. 2014) with accuracy better than 1-mm for the CoP position measurement (Good Balance System User Manual). The CoP position was calculated via the Good Balance Software (Metitur, Jyväskylä, Finland) which uses the weighted arithmetic mean between the vertical force measured by four sensors and their corresponding position: one in each corner of the force-plate and the last one in the centroid of the force-plate (Fig. 1). The rationale for using the tandem position for the feet was based in previous studies showing that greater pain effects are presented when posture is challenged (Hirata et al. 2013). This was important to ensure that postural stability adaptations due to pain could be observed. Therefore, subjects were asked to stand in tandem position, to increase postural challenge during the tasks, with the right leg behind (Fig. 1), arms hanging relaxed alongside the body, and were instructed to maintain balance while looking forward. Tape markers were placed on the force plate to ensure that the same foot position was maintained through all conditions. During

the assessment of postural control, subjects were instructed to look forward at a target positioned at eye-level approximately 45-cm from the subjects to minimize the influence of the target distance on postural sway (Kapoula and Lê 2006). CoP records were made under eight experimental conditions, depending on the type of injection (control or painful), the dual-task (counting forward or counting backward as the less and more challenging tasks, respectively), before (pre-injection) and immediately after the injection. The counting forward task consisted of adding one and the counting backward was performed by subtracting three, beginning from a random number. The total number of answers and the number of correct answers during each trial were recorded. The order of the injections and the order of the tasks were randomized, with the same number of subjects receiving the hypertonic or isotonic injections first.

The experiment always followed the same order for all participants: (i) CoP measurement while performing the first randomly assigned task (cognitive task 1 or 2) over 60-s (pre-injection 1); (ii) 1-min rest; (iii) CoP measurement over 60-s while performing the second randomly assigned task (cognitive task 1 or 2) over 60-s (pre-injection 2); (iv) injections of the first saline solution (painful or control) into vastus medialis (VM) and vastus lateralis (VL) muscles; (v) assessment of pain intensity by visual analogue scale (VAS); (vi) CoP measurement over 60-s while performing task A; (vii) collecting VAS scores of the pain intensity and 1-min rest; (viii) CoP measurement over 60-s while performing task B; (ix) collecting VAS scores of the pain intensity. After the final step, the pain VAS scores were taken each minute until the pain had subsided which was followed by a 5-min break. Following the break, all steps of the experiment were performed again with the injection of the other saline solution, including new pre-injection CoP recordings. Before each CoP measurement, all subjects confirmed that no tiredness or other problems were presented. The duration of the CoP measurements were performed according to guidelines proposed by the International Society for Posture and Gait Research (Scoppa et al. 2013). Fig. 2 summarizes the study procedures along time.

### 3.3. Experimental muscle pain

Before the experiment all subjects were instructed about the nature and effects of the injections,

1  
2  
3  
4  
5  
6  
7  
8 and that one type of injection would be painful while the other would be a non-painful stimulus, although  
9 they would not know which kind of injection they would be receiving. Pain was induced through  
10 intramuscular injection of 1-mL of 6% sterile hypertonic saline solution or as a control condition 1-mL of  
11 isotonic (0.9%) saline solution (Graven-Nielsen et al. 1997; Farina 2003; Schulte et al. 2004; Falla et al. 2006).  
12  
13 The injections were performed with a 2-mL syringe with a disposable needle (27G, 40-mm) into right VM  
14 muscle and right VL muscle. Both injections locations were marked to ensure that they were applied  
15 approximately in the same location. The VM muscle injection was performed 5-cm proximal and 5-cm medial  
16 to the medial corner of the patella (Shiozawa et al. 2013), and in the VL muscle, injections were performed  
17 at two thirds of the distance from the anterior spina iliaca to the lateral side of the patella (Fig. 3). The depth  
18 of the injection was determined by an ultrasound scanner (LOGIQ™ S7, General Electric, USA). This pain  
19 model has been successfully used previously to mimic knee-related pain during quiet standing tasks  
20 providing moderate pain intensities for approximately five minutes (Hirata et al. 2011). Hypertonic saline  
21 injections have been shown to activate nociceptors around the injected site (Mense 1993) whereas the 0.9%  
22 isotonic saline injections have induced little or no pain during postural control tasks similar to the one used  
23 in the present study (Hirata et al. 2010, 2011, 2013).  
24  
25 2.4. Assessment of pain intensity  
26  
27 The subjects were asked to rate the pain intensity using a 10-cm VAS from 0-cm to 10-cm (0-cm  
28 means “no pain” and 10-cm means “maximum pain”) immediately after the injections and after each balance  
29 measurement. Therefore, three VAS scores were obtained for each set of experiments (balance  
30 measurements after isotonic injection and balance measurements after hypertonic injection, respectively;  
31 Fig. 2), and the mean values of the three VAS scores were considered as the pain intensity after each injection  
32 paradigm. Additionally, following each set of experiments subjects were asked to indicate the overall pain  
33 areas during the trials on a body chart and to respond the McGill Pain Questionnaire (Melzack 1975). The  
34 area of pain was extracted from the body charts with VistaMetrix 1.38 software. The pain rating index based

Field Code Changed

Formatted: Danish

Formatted: Danish

1  
2  
3  
4  
5  
6  
7  
8 on the rank values of the words chosen within each category (sensory, affective, evaluative and  
9 miscellaneous) from McGill Pain Questionnaire were obtained and the score for each category, as well as  
10  
11 The total pain rating index were determined as the sum of the ranked values of the words (Melzack 1975).  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Results

### ~~3.1. Experimental muscle pain and cognitive task performance~~

#### ~~3.1. Area and amplitude of perceived pain'~~

Fig. 4 shows the reported pain areas following both isotonic and hypertonic injections. Pain was present in the anterior and lateral portions of the thigh after both isotonic and hypertonic injections, being more concentrated in the lower half of the thigh after the isotonic injections. The hypertonic saline injections induced higher pain area (mean area  $\pm$  SD: isotonic =  $518.6 \pm 690.6$  au; hypertonic =  $1659.3 \pm 1574.0$  au;  $P=0.003$ ) and higher VAS scores (mean score  $\pm$  SD: isotonic =  $0.9 \pm 1.1$  cm; hypertonic =  $4.7 \pm 1.7$  cm;  $P<0.001$ ) than isotonic saline injections. Table 1 shows the scores for each class of words from McGill Pain Questionnaire and the pain rating index. Subjects presented a higher total pain rating index and scored higher in all the categories, with the exception of the affective class, after the hypertonic injections ( $P<0.05$ ).

#### ~~3.2. Cognitive task performance~~

~~3.2.~~ Only for the analysis of the cognitive task performance, one subject was not included due to problems in the answers recording. The total number of answers and the number of correct answers decreased during backwards counting conditions compared with forwards counting despite the injection effect (significant main effect for *task factor*; Table 2).

### ~~3.3. Center of pressure~~

#### *Effect of experimental pain in CoP variables*

There were no statistical differences between the different conditions for the factor *injection* on any of the CoP variables (Table 3).

#### *Effect of cognitive task in CoP variables*

Formatted: Justified, Indent: First line: 0.49"

Formatted: Font: Italic

Formatted: Indent: First line: 0"

Formatted: Font: 12 pt, Italic

Formatted: Indent: First line: 0"

Formatted: Font: 12 pt, Italic

Formatted: Font: Italic

Formatted: Normal, Justified, No bullets or numbering

Formatted: Normal, Justified, No bullets or numbering

Formatted: Font: 12 pt, Italic

A main effect of *task* was found for the CoP AP-velocity ( $F=5.82$ ;  $P=0.028$ ), showing that there was an increased AP-velocity during the counting backwards task compared to the counting forwards task, regardless the type of injection (Table 3).

#### *Effect of the interaction between experimental pain and cognitive task in CoP variables*

An interaction effect was found between *injection* and *task* factors for CoP total area and CoP ML-velocity (CoP total  $F=7.78$ ,  $P=0.049$ ; CoP ML  $F=4.69$ ,  $P=0.021$ ) (Table 3). Post-hoc comparisons showed that both variables decreased after the hypertonic injection in comparison to the condition with isotonic injection when subjects were counting forward (Bonferroni:  $P = 0.010$  for total area;  $P = 0.015$  for ML-velocity). After the hypertonic injection, CoP total area increased when subjects were counting backwards in comparison to when they were counting forwards (Bonferroni:  $P = 0.019$ ). ML-velocity showed differences between the different cognitive tasks also after the injection of hypertonic solution, with a smaller decrease of ML-velocity while counting backwards (Bonferroni:  $P = 0.049$ ).

#### **4. Discussion**

The present study aimed at quantifying how postural stability, represented by CoP sway (velocity and area of displacement) and CoP complexity (CoP SaEn), is modified during experimental pain while performing a cognitive task. The main results showed that the kind of cognitive task did not interfere with postural stability in the absence of pain. Experimental pain around the knee joint reduced CoP sway but did not affect CoP complexity during the performance of an easier cognitive task. During experimentally induced pain, the performance of a difficult cognitive task increased CoP sway but did not change CoP complexity.

##### *Pain intensity and counting performance*

The subjects showed higher pain intensity for the hypertonic saline injection and a larger pain area compared with the isotonic saline injection, as expected, indicating that experimental pain occurred (Hirata et al. 2011). The McGill pain questionnaire indicated that hypertonic saline was perceived more impairing

than the isotonic injection in all subscales except for the affective one. It is important to note that during isotonic injections subjects rated pain around 1/10, which cannot be classified as a totally pain free condition. Counting performance requires the use of cognitive process which relies on the working memory of the subject (Lemaire 1996), impairing motor output performance when executed simultaneously with a motor task (Vuillerme and Nafati 2007). Seminowicz and Davis (2007) showed that subjects are able to maintain performance of difficult cognitive task while experiencing different levels of pain. In this study, the painful condition did not affect the counting performance while performing a motor task (standing still) indicating that healthy subjects are able to engage multiple tasks (motor and cognitive) during pain without compromising performance. This suggests that sufficient cognitive resources were available to manage the cognitive process of counting forwards or backwards despite the interpretation of painful stimuli and the postural control task (Eccleston et al. 1999). Finally, education level is associated with both motor and perceptual performance, where higher education level is associated with better performance (Voos et al. 2015). Since our subjects were all university students, we believe that bias due to education level did not affect the present results.

#### Effect of cognitive tasks on postural stability

Our first initial hypothesis, that (i) the kind of cognitive task (more or less demanding) in a non-painful condition would not interfere with CoP sway or CoP complexity, was confirmed. The factor task affected the CoP anterior-posterior velocity, indicating an increased velocity during the execution of the more difficult task (counting backwards) in comparison to the easier task (counting ~~backwards~~forward). Nevertheless, the CoP SaEn was not affected by the kind of the performed cognitive task. These results indicate that enough cognitive resources were available to overcome the demands of both cognitive and postural tasks, which was expected since they were young individuals without any sensory-motor alterations.

#### Effect of experimental knee-related pain on postural stability

Our second initial hypothesis, that (ii) experimental pain would increase CoP sway and decrease CoP complexity was not confirmed since the type of saline solution injected did not affect the CoP variables. However, even though the factor *injection* did not show statistical differences between the different conditions for any of the studied CoP variables, there was a difference between total area and ML-velocity between the control and the painful condition when the subjects were counting forwards, i.e., in conditions where the kind of cognitive task performed was the same. Interestingly, during the counting forward, the type of injection resulted significant changes in postural sway (total area and ML-velocity) in opposite directions: positive values of the difference between pre-injection and after injection of the isotonic solution, whereas after the injection of the hypertonic solution both variables showed negative values. Additionally, no significant changes were observed in the structural variability of the CoP signal. This is contrary to the initial hypothesis, where an increase in postural sway and a decrease in structural variability during painful conditions were expected. It is also in contrast with previous findings (Mazaheri et al. 2013) but may relate to the different position of the feet used in this study, which affects the postural sway (Day et al. 1993). The tandem feet position adopted allows less displacement of the CoP due to the limited base of support compared to side-by-side feet position, since if the subjects increase the CoP amplitude they may fall (Day et al. 1993). This also may reflect a voluntary strategy, requiring a greater amount of cognitive resources and attention (Morasso and Sanguineti 2002), attempting to avoid large excursions of the body and consequent loss of balance. For the current study, this might indicate that the subjects prioritized the balance task over the other tasks, also known as *posture first strategy* (Vuillerme and Nafati 2007). The subjects were able to reduce the postural sway without compromising the counting performance during the easy cognitive task, suggesting that the available cognitive resource was sufficient to perform the less challenging cognitive task without compromising postural stability. Therefore, these results indicate that healthy subjects have the capacity to perform easy cognitive tasks while ensuring postural stability (Siu and Woollacott 2007). Reducing postural sway might reflect a motor strategy available for healthy subjects to avoid excessive



translation of the body, which could lead to balance loss (Winter 1995). This strategy was also observed during the control injection while counting backwards, probably indicating that a high cognitive load seems to be interpreted as a treat to postural stability. An alternative explanation for the contrast between the present study and the previous studies with pain patients showing larger postural sway (Schulte et al. 2004; Levinger et al. 2016) might be the pain model used that is not a complete proxy to the impaired pain patients' sensory-motor system.

Interactions between pain and cognitive load on postural stability

Our initial third hypothesis, that (iii) the presence of experimental pain would increase CoP sway and decrease CoP complexity only when performing a difficult cognitive task was partially confirmed since CoP sway increased during pain under a difficult cognitive task, but the CoP complexity did not change. ANOVA results showed an interaction between the task and injection factors for total area and ML-velocity. After the hypertonic injection CoP total area increased and CoP ML-velocity decreased less while counting backwards in comparison to counting forwards condition, corroborating our hypothesis. ANOVA results also showed an effect of the task factor on AP-velocity with post-hoc comparisons showing a difference only during the hypertonic injection condition: while counting backwards AP-velocity also increased. Altogether these results show that CoP sway increases when performing a more demanding cognitive task in the presence of experimental pain. This might reflect an interference with the information-processing capacity and an attention disruption from both postural control and cognitive task (Eccleston et al. 1999). Previous studies suggest that disruptions of sensory information lead to worsening of proprioception in the affected area (Matre et al. 2002), further impairing postural sway (Hirata et al. 2010, 2011). The results indicate that the posture first strategy (Vuillerme and Nafati 2007) found during the easy cognitive task during pain is no longer feasible when a difficult cognitive task is performed during painful conditions. The increased cognitive load in painful conditions seems to impair the motor performance maybe due to insufficient cognitive resource to simultaneously maintain postural stability (which requires significant amount of attention

(Morasso and Sanguineti 2002)) and execute a difficult cognitive task. These results might have important new implications in understanding the mechanisms related to fall accidents. Postural stability in daily life activities is usually performed in combination with additional tasks, for example, walking in a busy slippery sidewalk. These daily life activities involves simultaneously competition for the cognitive resources available (Woollacott and Shumway-Cook 2002) to evaluate the environment constrains in order to promote the best motor strategy (Winter 1995). Our present results indicate that, if the subject performs a challenging postural task in pain, his/her capacity for maintain balance while exposed to a difficult cognitive task is suboptimal, which could increase the likelihood of losing balance.

The complexity of postural sway did not show any differences between the experimental conditions. This result is contrary to the literature finding that young healthy subjects present a more regular and less automatic postural sway (decreased CoP SaEn) when the motor task is more difficult (e. g. standing with eyes closed) and more irregular postural sway and more automatic postural sway (increased CoP SaEn) when a cognitive task is added (Donker et al. 2007; Stins et al. 2009). The fact that the cognitive task did not interfere with CoP complexity may be due to the nature of both motor (standing in tandem position) and cognitive subtraction calculus) tasks used in the experimental setup that did not interfere with the automaticity of postural control. Besides that, pain also did not affect CoP complexity, showing that experimental knee-related pain did not compromise the coupling between the components of the system responsible for balance in the current experimental setup. Future studies should investigate the interaction between pain, cognition and on CoP complexity with different motor and cognitive demands, in addition to different populations.

Despite interesting results regarding the effects of cognitive tasks in postural control during pain, the relevance of the findings for clinical populations should be interpreted with care. The experimental pain model used here is convenient to assess the effect of pain without the interference of potential structural or pathologies. However, extrapolating the current findings to an older population can only be done to some

degree. Additionally, chronic pain patients may also suffer from depressive symptoms (Bair et al. 2003) or anxiety (McWilliams et al. 2003), which might increase cognitive load (Nebes et al. 2001). Furthermore, cognitive impairments are often found in chronic pain patients, decreasing the possibility to maintain performance of two or more concurrent tasks (Brauer et al. 2004), as opposed to what was observed in this study where young healthy subjects were recruited. Also, there was no recording of postural sway without any cognitive task. This would have allowed comparisons with a condition where neither pain nor cognitive tasks were influencing postural sway, and could have reduced type 2 errors given that multiple CoP variables were analyzed in the study. Thus, it can be considered a limitation to our interpretations.

## 5. Conclusions

Pain and cognitive task interfered on postural stability, changing its patterns. During the performance of a simple cognitive task, pain reduced postural sway, while during the performance of a more demanding cognitive task, postural sway was increased in young healthy subjects. Since our subjects were young healthy subjects, the direct translation of the present results to patients suffering from pain should be done with caution. However, these results may suggest that rehabilitation approaches should take into account that pain not only affects directly the motor system, but may occupy cognitive resources, potentially resulting in poorer performance when performing rehabilitation exercises. Additionally, rehabilitation strategies using both motor and cognitive resources need further investigation to outline the effect of interaction between pain and cognition on the performance during activities of daily life in patients.

## 6. Compliance with ethical standards

**Funding:** Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121). The authors thank the State of São Paulo Research Foundation (FAPESP) for the Suda scholarship (FAPESP 2013/06123-7, 2015/00214-6).

**Conflict of Interest:** The authors declare that they have no conflict of interest.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## REFERENCES

- Andersson G, Hagman J, Talianzadeh R, et al (2002) Effect of cognitive load on postural control. *Brain Res Bull* 58:135–9
- Bair MJ, Robinson RL, Katon W, Kroenke K (2003) Depression and Pain Comorbidity. *Arch Intern Med* 163:2433. doi: 10.1001/archinte.163.20.2433
- Brauer SG, Broome A, Stone C, et al (2004) Simplest tasks have greatest dual task interference with balance in brain injured adults. *Hum Mov Sci* 23:489–502. doi: 10.1016/j.humov.2004.08.020
- Bronstein AM, Buckwell D (1997) Automatic control of postural sway by visual motion parallax. *Exp Brain Res* 113:243–248. doi: 10.1007/BF02450322
- Day BYBL, Steiger MJ, Thompson PD, Marsden CD (1993) Human Body Motion When Standing : Implications for. *J Physiol* 469:479–499
- Donker SF, Roerdink M, Greven AJ, Beek PJ (2007) Regularity of center-of-pressure trajectories depends on the amount of attention invested in postural control. *Exp Brain Res* 181:1–11. doi: 10.1007/s00221-007-0905-4
- Duarte M, Sternad D (2008) Complexity of human postural control in young and older adults during prolonged standing. *Exp Brain Res* 191:265–276. doi: 10.1007/s00221-008-1521-7
- Eccleston C, Baeyens F, Helen P, et al (1999) Pain Demands Attention : A Cognitive-Affective Model of the Interruptive Function of Pain. 125:356–366
- Era P, Sainio P, Koskinen S, et al (2006) Postural balance in a random sample of 7,979 subjects aged 30 years and over. *Gerontology* 52:204–213. doi: 10.1159/000093652
- Etemadi Y, Salavati M, Arab AM, Ghanavati T (2016) Balance recovery reactions in individuals with recurrent nonspecific low back pain: Effect of attention. *Gait Posture* 44:123–127. doi: 10.1016/j.gaitpost.2015.11.017
- Falla D, Farina D, Dahl MK, Graven-Nielsen T (2006) Muscle pain induces task-dependent changes in cervical agonist/antagonist activity. *J Appl Physiol* 102:601–609. doi: 10.1152/jappphysiol.00602.2006
- Farina D (2003) Effect of Experimental Muscle Pain on Motor Unit Firing Rate and Conduction Velocity. *J Neurophysiol* 91:1250–1259. doi: 10.1152/jn.00620.2003
- Fewster KM, Gallagher KM, Howarth SH, Callaghan JP (2017) Low back pain development differentially influences centre of pressure regularity following prolonged standing. *Gait Posture*. doi: 10.1016/j.gaitpost.2017.06.005
- Fraizer E V., Mitra S (2008) Methodological and interpretive issues in posture-cognition dual-tasking in upright stance. *Gait Posture* 27:271–279. doi: 10.1016/j.gaitpost.2007.04.002
- Graven-Nielsen T, Arendt-Nielsen L, Svensson P, Jensen TS (1997) Quantification of local and referred muscle pain in humans after sequential i.m. injections of hypertonic saline. *Pain* 69:111–7
- Ha H, Cho K, Lee W (2014) Reliability of the Good Balance System® for Postural Sway Measurement in Poststroke Patients. *J Phys Ther Sci* 26:121–124. doi: 10.1589/jpts.26.121
- Harbourne RT, Stergiou N (2003) Nonlinear analysis of the development of sitting postural control. *Dev Psychobiol* 42:368–377. doi: 10.1002/dev.10110
- Hirata RP, Arendt-Nielsen L, Graven-Nielsen T (2010) Experimental calf muscle pain attenuates the postural

- stability during quiet stance and perturbation. *Clin Biomech* 25:931–937. doi: 10.1016/j.clinbiomech.2010.06.001
- Hirata RP, Ervilha UF, Arendt-Nielsen L, Graven-Nielsen T (2011) Experimental muscle pain challenges the postural stability during quiet stance and unexpected posture perturbation. *J Pain* 12:911–919. doi: 10.1016/j.jpain.2011.02.356
- Hirata RP, Jørgensen TS, Rosager S, et al (2013) Altered Visual and Feet Proprioceptive Feedbacks during Quiet Standing Increase Postural Sway in Patients with Severe Knee Osteoarthritis. *PLoS One* 8:1–8. doi: 10.1371/journal.pone.0071253
- Huxhold O, Li SC, Schmiedek F, Lindenberger U (2006) Dual-tasking postural control: Aging and the effects of cognitive demand in conjunction with focus of attention. *Brain Res Bull* 69:294–305. doi: 10.1016/j.brainresbull.2006.01.002
- Kahneman D (1973) *Attention and effort*. Prentice-Hall
- Kapoula Z, Lê TT (2006) Effects of distance and gaze position on postural stability in young and old subjects. *Exp Brain Res* 173:438–445. doi: 10.1007/s00221-006-0382-1
- Knoop J, Van Der Leeden M, Van Der Esch M, et al (2012) Association of lower muscle strength with self-reported knee instability in osteoarthritis of the knee: Results from the Amsterdam Osteoarthritis Cohort. *Arthritis Care Res* 64:38–45. doi: 10.1002/acr.20597
- Kuczyński M, Szymańska M, Bieć E (2011) Dual-task effect on postural control in high-level competitive dancers. *J Sports Sci* 29:539–545. doi: 10.1080/02640414.2010.544046
- Larivière C, Butler H, Sullivan MJL, Fung J (2013) An exploratory study on the effect of pain interference and attentional interference on neuromuscular responses during rapid arm flexion movements. *Clin J Pain* 29:265–275. doi: 10.1097/AJP.0b013e318250ed6f
- Lemaire P (1996) The Role of Working Memory Resources in Simple Cognitive Arithmetic. *Eur J Cogn Psychol* 8:73–104. doi: 10.1080/095414496383211
- Levinger P, Nagano H, Downie C, et al (2016) Biomechanical balance response during induced falls under dual task conditions in people with knee osteoarthritis. *Gait Posture* 48:106–112. doi: 10.1016/j.gaitpost.2016.04.031
- Liston MB, Bergmann JH, Keating N, et al (2014) Postural prioritization is differentially altered in healthy older compared to younger adults during visual and auditory coded spatial multitasking. *Gait Posture* 39:198–204. doi: 10.1016/j.gaitpost.2013.07.004
- Madeleine P, Nielsen M, Arendt-Nielsen L (2011) Characterization of postural control deficit in whiplash patients by means of linear and nonlinear analyses - A pilot study. *J Electromyogr Kinesiol* 21:291–297. doi: 10.1016/j.jelekin.2010.05.006
- Manor B, Costa MD, Hu K, et al (2010) Physiological complexity and system adaptability: evidence from postural control dynamics of older adults. *J Appl Physiol* 109:1786–1791. doi: 10.1152/jappphysiol.00390.2010
- Matre D, Arendt-Nielsen L, Knardahl S (2002) Effects of localization and intensity of experimental muscle pain on ankle joint proprioception. *Eur J Pain* 6:245–260. doi: 10.1053/eujp.2002.0332
- Mazaheri M, Coenen P, Parnianpour M, et al (2013) Low back pain and postural sway during quiet standing with and without sensory manipulation: A systematic review. *Gait Posture* 37:12–22. doi:

- 10.1016/j.gaitpost.2012.06.013
- Mazaheri M, Heidari E, Mostamand J, et al (2014) Competing effects of pain and fear of pain on postural control in low back pain? *Spine (Phila Pa 1976)* 39:E1518–E1523. doi: 10.1097/BRS.0000000000000605
- McWilliams LA, Cox BJ, Enns MW (2003) Mood and anxiety disorders associated with chronic pain: an examination in a nationally representative sample. *Pain* 106:127–33
- Melzack R (1975) The McGill Pain Questionnaire: major properties and scoring methods. *Pain* 1:277–99
- Mense S (1993) Nociception from skeletal muscle in relation to clinical muscle pain. *Pain* 54:241–89
- Morasso PGP, Sanguineti V (2002) Ankle muscle stiffness alone cannot stabilize balance during quiet standing. *J Neurophysiol* 88:2157–2162. doi: 10.1152/jn.00719.2001
- Neves RD, Butters MA, Houck PR, et al (2001) Dual-task performance in depressed geriatric patients. *Psychiatry Res* 102:139–151. doi: 10.1016/S0165-1781(01)00244-X
- Pellecchia GL (2003) Postural sway increases with attentional demands of concurrent cognitive task. *Gait Posture* 18:29–34. doi: 10.1016/S0966-6362(02)00138-8
- Peterka RJ (2003) Dynamic Regulation of Sensorimotor Integration in Human Postural Control. *J Neurophysiol* 91:410–423. doi: 10.1152/jn.00516.2003
- Richman JS, Moorman JR (2000) Physiological time-series analysis using approximate entropy and sample entropy. *Physiological time-series analysis using approximate entropy and sample entropy. Am Physiol Soc*
- ~~Roerdink M, De Haart M, Daffertshofer A, et al (2006) Dynamical structure of center-of-pressure trajectories in patients recovering from stroke. *Exp Brain Res* 174:256–269. doi: 10.1007/s00221-006-0441-7~~
- Schulte E, Ciubotariu A, Arendt-Nielsen L, et al (2004) Experimental muscle pain increases trapezius muscle activity during sustained isometric contractions of arm muscles. *Clin Neurophysiol* 115:1767–1778. doi: 10.1016/j.clinph.2004.03.005
- Scoppa F, Capra R, Gallamini M, Shiffer R (2013) Clinical stabilometry standardization. Basic definitions - Acquisition interval - Sampling frequency. *Gait Posture* 37:290–292. doi: 10.1016/j.gaitpost.2012.07.009
- Seminowicz DA, Davis KD (2007) Interactions of pain intensity and cognitive load: The brain stays on task. *Cereb Cortex* 17:1412–1422. doi: 10.1093/cercor/bhl052
- Sherafat S, Salavati M, Takamjani IE, et al (2014) Effect of dual-tasking on dynamic postural control in individuals with and without nonspecific low back pain. *J Manipulative Physiol Ther* 37:170–179. doi: 10.1016/j.jmpt.2014.02.003
- Shiozawa S, Hirata RP, Graven-Nielsen T (2013) Reorganised anticipatory postural adjustments due to experimental lower extremity muscle pain. *Hum Mov Sci* 32:1239–1252. doi: 10.1016/j.humov.2013.01.009
- Siu KC, Woollacott MH (2007) Attentional demands of postural control: The ability to selectively allocate information-processing resources. *Gait Posture* 25:121–126. doi: 10.1016/j.gaitpost.2006.02.002
- Slifkin AB, Newell KM (1999) Noise, information transmission, and force variability. *J Exp Psychol Hum*

- Percept Perform 25:837–851. doi: 10.1037//0096-1523.25.3.837
- Søndergaard KHE, Olesen CG, Søndergaard EK, et al (2010) The variability and complexity of sitting postural control are associated with discomfort. *J Biomech* 43:1997–2001. doi: 10.1016/j.jbiomech.2010.03.009
- Stins JF, Michielsen ME, Roerdink M, Beek PJ (2009) Sway regularity reflects attentional involvement in postural control: Effects of expertise, vision and cognition. *Gait Posture* 30:106–109. doi: 10.1016/j.gaitpost.2009.04.001
- Swan L, Otani H, Loubert P V. (2007) Reducing postural sway by manipulating the difficulty levels of a cognitive task and a balance task. *Gait Posture* 26:470–474. doi: 10.1016/j.gaitpost.2006.11.201
- Vaillancourt DE, Newell KM (2002) Changing complexity in human behavior and physiology through aging and disease. *Neurobiol Aging* 23:1–11. doi: 10.1016/S0197-4580(02)00052-0
- Vaillancourt DE, Newell KM (2000) The dynamics of resting and postural tremor in Parkinson’s disease. *Clin Neurophysiol* 111:2046–2056. doi: 10.1016/S1388-2457(00)00467-3
- Van Daele U, Hagman F, Truijen S, et al (2010) Decrease in postural sway and trunk stiffness during cognitive dual-task in nonspecific chronic low back pain patients, performance compared to healthy control subjects. *Spine (Phila Pa 1976)* 35:583–589. doi: 10.1097/BRS.0b013e3181b4fe4d
- Van Ryckeghem DM, Van Damme S, Eccleston C, Crombez G (2018) The efficacy of attentional distraction and sensory monitoring in chronic pain patients: A meta-analysis. *Clin Psychol Rev* 59:16–29. doi: 10.1016/j.cpr.2017.10.008
- Veldhuijzen DS, Kenemans JL, De Bruin CM, et al (2006) Pain and attention: Attentional disruption or distraction? *J Pain* 7:11–20. doi: 10.1016/j.jpain.2005.06.003
- Voos MC, Piemonte MEP, Castelli LZ, et al (2015) Association between Educational Status and Dual-Task Performance in Young Adults. *Percept Mot Skills* 120:416–437. doi: 10.2466/22.PMS.120v18x8
- Vuillerme N, Nafati G (2007) How attentional focus on body sway affects postural control during quiet standing. *Psychol Res* 71:192–200. doi: 10.1007/s00426-005-0018-2
- Winter DA (1995) A. B. C. (Anatomy, Biomechanics, Control) of Balance During Standing and Walking. Waterloo Biomechanics, Ontario
- Woollacott M, Shumway-Cook A (2002) Attention and the control of posture and gait: A review of an emerging area of research. *Gait Posture* 16:1–14. doi: 10.1016/S0966-6362(01)00156-4

### Figure captions

**Fig 1** Schematic drawing representing the force platform size, sensor locations, and the tandem position of the subjects during the experiment

**Fig 2** Study design overview: pain assessments were performed immediately after each injection and each balance measurement; the order of the saline injections was randomized in a balanced way

**Fig 3** Injections sites for vastus lateralis muscle, performed at two thirds of the distance from the anterior spina iliaca (a) to the lateral side of the patella (b); and for the vastus medialis muscle, performed 5 cm proximal and 5 cm medial to the medial corner of the patella (c),

**Fig 4** Representation of the experimental pain distribution reported areas after isotonic (top, blue in the online version) and hypertonic (bottom, red in the online version saline injections (A); the individual distributions are superimposed in the anatomical drawings



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

585 **EXPERIMENTAL KNEE-RELATED PAIN ENHANCES ATTENTIONAL INTERFERENCE ON POSTURAL CONTROL**

586 Eneida Yuri Suda<sup>1</sup>, Rogerio Pessoto Hirata<sup>2\*</sup>, Thorvaldur Palsson<sup>2</sup>, Nicolas Vuillerme<sup>3</sup>, Isabel C N Sacco<sup>1</sup>,  
587 Thomas Graven-Nielsen<sup>4</sup>

588  
589 <sup>1</sup> Laboratory of Biomechanics of Human Movement, Dept. Physical Therapy, Speech and Occupational  
590 <sup>2</sup> Therapy, School of Medicine, University of Sao Paulo, Sao Paulo, Brazil  
591 <sup>3</sup> Department of Health Science and Technology, SMI, Faculty of Medicine, Aalborg University, Aalborg,  
592 <sup>4</sup> Denmark  
593 <sup>5</sup> Univ. Grenoble-Alpes, EA AGEIS, Grenoble, France & Institut Universitaire de France  
594 <sup>6</sup> Center for Neuroplasticity and Pain (CNAP), SMI, Department of Health Science and Technology, Aalborg  
595 <sup>7</sup> University, Denmark

596  
597 Original article for: European Journal of Applied Physiology

598 Number of Pages: 24

599 Number of words: 5009

600 Number of figures: 4

601 Number of tables: 3

602 Abstract: 249

603 **\*Corresponding author:**

604 Associate Professor Rogerio Pessoto Hirata, Ph.D.  
605 Center for Sensory Motor Interaction, SMI, Department of Health Science and Technology, Aalborg University,  
606 Aalborg, Denmark  
607 Department of Health Science and Technology  
608 Aalborg University, Fredrik Bajersvej 7D-3  
609 9220 Aalborg E, Denmark  
610 E-mail: [rirata@hst.aau.dk](mailto:rirata@hst.aau.dk)

611  
612 **Acknowledgement**

613 Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation  
614 (DNRF121). The authors thank the State of São Paulo Research Foundation (FAPESP) for the Suda scholarship  
615 (FAPESP 2017/15449-4, 2015/00214-6).

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Abstract

**Purpose:** To quantify how postural stability is modified during experimental pain while performing different cognitively demanding tasks.

**Methods:** Sixteen healthy young adults participated in the experiment. Pain was induced by intramuscular injection of hypertonic saline solution (1mL, 6%) in both vastus medialis and vastus lateralis muscles (0.9% isotonic saline was used as control). The participants stood barefoot in tandem position for one minute on a force plate. Center of pressure (CoP) was recorded before and immediately after injections, while performing two cognitive tasks: (i) counting forwards by adding one; (ii) counting backwards by subtracting three. CoP variables – total area of displacement, velocity in anterior-posterior (AP-velocity) and medial-lateral (ML-velocity) directions, and CoP sample entropy in anterior-posterior and medial-lateral directions were displayed as the difference between the values obtained after and before each injection and compared between tasks and injections.

**Results:** CoP total area ( $-84.5 \pm 145.5$  vs.  $28.9 \pm 78.5$  cm<sup>2</sup>) and ML-velocity ( $-1.71 \pm 2.61$  vs.  $0.98 \pm 1.93$  cm/s) decreased after the painful injection vs. Control injection while counting forward ( $P < 0.05$ ). CoP total area ( $112.8 \pm 53.9$  vs.  $-84.5 \pm 145.5$  cm<sup>2</sup>), ML-velocity ( $-0.34 \pm 1.92$  vs.  $-1.71 \pm 2.61$  cm/s) and AP-velocity ( $1.07 \pm 2.35$  vs.  $-0.39 \pm 1.82$  cm/s) increased while counting backwards vs. forwards after the painful injection ( $P < 0.05$ ).

**Conclusion:** Pain interfered with postural stability according to the type of cognitive task performed, suggesting that pain may occupy cognitive resources, potentially resulting in poorer balance performance.

**Keywords:** postural stability, center of pressure, attention, distraction, pain

			1
			2
			3
			4
			5
			6
			7
637		<b>List of abbreviations</b>	8
638			9
			10
639		ANOVA	Analysis of variance
			11
640		au	Arbitrary units
			13
641		CoP	Center of pressure
			15
642		SaEn	Sample entropy
			17
643		SD	Standard deviation
			19
644		VAS	Visual analogue scale
			21
645		VM	Vastus medialis
			23
646		VL	Vastus lateralis
			24
647			25
			26
			27
			28
			29
			30
			31
			32
			33
			34
			35
			36
			37
			38
			39
			40
			41
			42
			43
			44
			45
			46
			47
			48
			49
			50
			51
			52
			53
			54
			55
			56
			57
			58
			59
			60
			61
			62
			63
			64
			65

## 1. Introduction

Controlling of upright posture requires a significant amount of attention to gather information from the body and the environment and to generate adapted and accurate muscle activation for postural control (Morasso and Sanguineti 2002). Although the majority of postural control is regulated via automatic neural processes (Bronstein and Buckwell 1997), higher cortical centers are significantly involved in processing sensory information to plan and execute the best motor strategy for postural control (Winter 1995). In daily life, postural control is challenging as several tasks simultaneously compete for the cognitive resources available (Woollacott and Shumway-Cook 2002), limited by the capacity of higher centers to process sensory information (Kahneman 1973). Therefore, sharing attentional resources may cause impairments in the performance of daily living activities (Brauer et al. 2004). For example, competition for cognitive resources during tasks involving postural stability results in body stability being prioritized over secondary tasks (Liston et al. 2014).

Dual tasks paradigms, where subjects perform an additional task during standing, are employed to quantify the extent to which attention is associated with postural control. Decreases in postural sway while performing a secondary task compared with control conditions have been reported (Andersson et al. 2002; Pellecchia 2003) whereby focusing the attention on standing as still as possible increased postural sway compared with conditions without similar instructions (Vuillerme and Nafati 2007). Altogether, these results suggest that postural control demands attention (Woollacott and Shumway-Cook 2002) and that simultaneous cognitive loading plays an important role in balance stability (Swan et al. 2007).

Although detrimental effects of cognitive loading on postural sway during unperturbed standing are more commonly reported for older adults and patients, studies using dual-task approaches in young subjects show controversial results (Huxhold et al. 2006; Fraizer and Mitra 2008). Young healthy subjects have probably more ability to allocate the attentional resources without sacrificing postural stability, showing that

a system without impairments prioritizes postural stability when dealing with dual-cognitive tasks (Siu and Woollacott 2007).

Subjects with pain demonstrate increased postural sway compared with controls (Hirata et al. 2011). A possible explanation for this finding is that the increased postural sway may relate to a disrupting effect of nociceptive stimuli on attention to other simultaneous non-nociceptive tasks (Eccleston et al. 1999), underlining that processing of nociceptive stimuli is cognitively demanding (Veldhuijzen et al. 2006). Thus, the execution of cognitive tasks during pain might interfere with postural control. Although previous studies have shown that patients with pain present impaired balance while performing a secondary cognitive task in comparison to health subjects (Van Daele et al. 2010; Larivière et al. 2013; Mazaheri et al. 2014; Sherafat et al. 2014; Etemadi et al. 2016; Levinger et al. 2016), it is not clear yet the isolate effect of pain since reduced muscle strength, reduced flexibility and degenerative changes at the affected segment also cause both stiffness and instability in patients suffering from chronic pain (Knoop et al. 2012). Therefore, further investigation of the interaction between pain, cognition and postural stability is warranted. This investigation is of particular interest for clinical practice since there are evidences that attention can be directed away from pain using some specific strategies (Van Ryckeghem et al. 2018). If selective attention could be directed away from the painful stimulus and modify the deleterious effect of muscle pain on postural control, these results could have important implications for clinical settings. Likewise, if the execution of cognitive tasks impairs postural control in the presence of pain, this should also be taken into account in rehabilitation context.

Considering that posture can be defined as the dynamic stability of a continuous moving body (Harbourne and Stergiou 2003; Madeleine et al. 2011), nonlinear analysis of the dynamic structure of the center of pressure (CoP) time series would contribute to understand the physiological complexity of posture by accessing motor patterns that would be implicit in the CoP variability. Sample entropy (SaEn) measures variations in the system output along time. Therefore, measures of physiological complexity of the postural

1  
2  
3  
4  
5  
6  
7  
8 sway during quiet standing may relate to the system functionality as they are defined as the capacity of  
9 generating adaptive answers to an ever-changing environment such as controlling posture (Manor et al.  
10  
11  
12 2010). SaEn provides a measure of “orderly structure” within the time series since it tests if there are any  
13 repeated patterns of various lengths, including the ones that are not repeated at regular intervals (Duarte  
14  
15 and Sternad 2008). So, the lower the SaEn values are, the higher the similarity and lesser the complexity in  
16  
17 the temporal series is (Richman and Moorman 2000).

18  
19 Complexity depends on the number of structural components of the system, the existing coupling  
20  
21 among these components and how this interaction is influenced by the intrinsic dynamic properties of the  
22  
23 system and the motor task demands (Vaillancourt and Newell 2002). Thus, if the presence of pain and the  
24  
25 execution of a cognitive task are both concurring with the attentional resources used in postural control,  
26  
27 then the coupling between the components of the system responsible for balance may be affected and,  
28  
29 consequently, the complexity of the postural sway is affected. Execution of a concurrent cognitive task  
30  
31 during standing increases the complexity of the postural sway, and this increase has been attributed to a  
32  
33 more automatized postural sway, when less attention is directed to the balance control (Donker et al. 2007;  
34  
35 Stins et al. 2009; Kuczyński et al. 2011). On the other hand, there is some evidence that the complexity of  
36  
37 postural control decreases with pain during sitting with increased perceived discomfort in healthy young  
38  
39 subjects (Søndergaard et al. 2010). Similar finding was reported in young subjects with transient acute  
40  
41 episode of low back pain during two continuous hours of standing, but without history of low back pain  
42  
43 (Fewster et al. 2017), showing a relation between the occurrence of pain and the decrease in CoP complexity.  
44  
45 Therefore, examining the complexity of postural sway in a dual task context and the effect of experimental  
46  
47 pain in this condition may improve the understanding of the decrease in postural stability (Levinger et al.  
48  
49 2016) and complexity (Fewster et al. 2017) that may exist as a result of pain in an otherwise healthy system.

50  
51 The aim of this study was to quantify how postural stability [CoP sway velocity and area of  
52  
53 displacement and complexity (CoP SaEn)], is modified during experimental pain while performing a cognitive  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

task. It was hypothesized that (i) the kind of cognitive task (more or less demanding) in a non-painful condition will not interfere with CoP sway or CoP complexity, since the system would have enough cognitive resources to overcome it; (ii) experimental pain will increase CoP sway and decrease CoP complexity, regardless the type of cognitive task performed; (iii) the presence of experimental pain while performing a difficult cognitive task will overload the cognitive resources and impair postural stability, increasing CoP sway and decreasing CoP complexity.

## 2. Methods

### 2.1. Subjects

Sixteen young adults, all university students, (to control for the effect of education level on multitasking performance (Voos et al. 2015)), participated in the experiment – 8 males (mean  $\pm$  SD: age =  $26.9 \pm 2.8$  years; body mass =  $74.9 \pm 13.8$  kg; height =  $1.76 \pm 0.08$  m) and 8 females (mean  $\pm$  SD: age =  $27.1 \pm 1.0$  years; body mass =  $68.8 \pm 5.2$  kg; height =  $1.68 \pm 0.06$  m). The exclusion criteria were body mass index above  $25 \text{ kg/m}^2$ , pregnancy, drug addiction, previous neurologic, musculoskeletal or mental illness, lack of ability to cooperate, current use of medications (e.g. analgesics, anti-inflammatory medicine), consumption of alcohol, caffeine, nicotine or painkillers 8 hours prior to the data collection, recent history of acute pain affecting the lower limb and/or trunk, past history of chronic pain conditions, participation in other pain trials throughout the study period. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the local Ethics Committee (N-20120077). This sample size was calculated to detect a minimum difference of 40% in the CoP area assuming type error 1 as 5% and power of 80% between the conditions before and after the induction of experimental pain. All participants gave signed informed consents prior to inclusion in the study.

### 2.2. Experimental protocol

Since in healthy individuals approximately 70% of the information used for controlling posture originates from proprioceptive systems (Peterka 2003), we controlled the effect of different footwear on postural control by asking the subjects to stand barefoot during the experiment. The participants stood on a triangular force plate that measures vertical forces (Good Balance System, Metitur, Jyväskylä, Finland; dimensions: equilateral triangle – 800-mm; sampling frequency: 50-Hz as suggested by the International Society for Posture and Gait Research Standardization Committee (Scoppa et al. 2013)). This is a valid and reliable system for postural sway measurements (Era et al. 2006; Ha et al. 2014) with accuracy better than 1-mm for the CoP position measurement (Good Balance System User Manual). The CoP position was calculated via the Good Balance Software (Metitur, Jyväskylä, Finland) which uses the weighted arithmetic mean between the vertical force measured by four sensors and their corresponding position: one in each corner of the force-plate and the last one in the centroid of the force-plate (Fig. 1). The rationale for using the tandem position for the feet was based in previous studies showing that greater pain effects are presented when posture is challenged (Hirata et al. 2013). This was important to ensure that postural stability adaptations due to pain could be observed. Therefore, subjects were asked to stand in tandem position, to increase postural challenge during the tasks, with the right leg behind (Fig. 1), arms hanging relaxed alongside the body, and were instructed to maintain balance while looking forward. Tape markers were placed on the force plate to ensure that the same foot position was maintained through all conditions. During the assessment of postural control, subjects were instructed to look forward at a target positioned at eye-level approximately 45-cm from the subjects to minimize the influence of the target distance on postural sway (Kapoula and Lê 2006). CoP records were made under eight experimental conditions, depending on the type of injection (control or painful), the dual-task (counting forward or counting backward as the less and more challenging tasks, respectively), before (pre-injection) and immediately after the injection. The counting forward task consisted of adding one and the counting backward was performed by subtracting three, beginning from a random number. The total number of answers and the number of correct answers



during each trial were recorded. The order of the injections and the order of the tasks were randomized, with the same number of subjects receiving the hypertonic or isotonic injections first.

The experiment always followed the same order for all participants: (i) CoP measurement while performing the first randomly assigned task (cognitive task 1 or 2) over 60-s (pre-injection 1); (ii) 1-min rest; (iii) CoP measurement over 60-s while performing the second randomly assigned task (cognitive task 1 or 2) over 60-s (pre-injection 2); (iv) injections of the first saline solution (painful or control) into vastus medialis (VM) and vastus lateralis (VL) muscles; (v) assessment of pain intensity by visual analogue scale (VAS); (vi) CoP measurement over 60-s while performing task A; (vii) collecting VAS scores of the pain intensity and 1-min rest; (viii) CoP measurement over 60-s while performing task B; (ix) collecting VAS scores of the pain intensity. After the final step, the pain VAS scores were taken each minute until the pain had subsided which was followed by a 5-min break. Following the break, all steps of the experiment were performed again with the injection of the other saline solution, including new pre-injection CoP recordings. Before each CoP measurement, all subjects confirmed that no tiredness or other problems were presented. The duration of the CoP measurements were performed according to guidelines proposed by the International Society for Posture and Gait Research (Scoppa et al. 2013). Fig. 2 summarizes the study procedures along time.

### 2.3. Experimental muscle pain

Before the experiment all subjects were instructed about the nature and effects of the injections, and that one type of injection would be painful while the other would be a non-painful stimulus, although they would not know which kind of injection they would be receiving. Pain was induced through intramuscular injection of 1-mL of 6% sterile hypertonic saline solution or as a control condition 1-mL of isotonic (0.9%) saline solution (Graven-Nielsen et al. 1997; Farina 2003; Schulte et al. 2004; Falla et al. 2006). The injections were performed with a 2-mL syringe with a disposable needle (27G, 40-mm) into right VM muscle and right VL muscle. Both injections locations were marked to ensure that they were applied approximately in the same location. The VM muscle injection was performed 5-cm proximal and 5-cm medial

to the medial corner of the patella (Shiozawa et al. 2013), and in the VL muscle, injections were performed at two thirds of the distance from the anterior spina iliaca to the lateral side of the patella (Fig. 3). The depth of the injection was determined by an ultrasound scanner (LOGIQ™ S7, General Electric, USA). This pain model has been successfully used previously to mimic knee-related pain during quiet standing tasks providing moderate pain intensities for approximately five minutes (Hirata et al. 2011). Hypertonic saline injections have been shown to activate nociceptors around the injected site (Mense 1993) whereas the 0.9% isotonic saline injections have induced little or no pain during postural control tasks similar to the one used in the present study (Hirata et al. 2010, 2011, 2013).

#### 2.4. Assessment of pain intensity

The subjects were asked to rate the pain intensity using a 10-cm VAS from 0-cm to 10-cm (0-cm means “no pain” and 10-cm means “maximum pain”) immediately after the injections and after each balance measurement. Therefore, three VAS scores were obtained for each set of experiments (balance measurements after isotonic injection and balance measurements after hypertonic injection, respectively; Fig. 2), and the mean values of the three VAS scores were considered as the pain intensity after each injection paradigm. Additionally, following each set of experiments subjects were asked to indicate the overall pain areas during the trials on a body chart and to respond the McGill Pain Questionnaire (Melzack 1975). The area of pain was extracted from the body charts with VistaMetrix 1.38 software. The pain rating index based on the rank values of the words chosen within each category (sensory, affective, evaluative and miscellaneous) from McGill Pain Questionnaire were obtained and the score for each category, as well as the total pain rating index were determined as the sum of the ranked values of the words (Melzack 1975).

#### 2.5. Data analysis

All variables for postural sway were calculated based on 50-s of the standing tasks, with the first and last 5-s from the original 60-s time series being excluded. The analyses were performed with Matlab R2016a software (Mathworks, Massachusetts, USA). The area fitted to 95% confidence interval of the CoP

displacement was calculated as representative of the total CoP area displacement (95% confidence interval ellipse), along with the CoP velocity in both directions (anterior-posterior and medial-lateral). The structural variability of the CoP was calculated by means of SaEn with the embedding dimension ( $m$ ) and the tolerance distance ( $r$ ) set to  $m=2$  and  $r=0.2 \times \text{SD}$  (Vaillancourt and Newell 2000). All CoP variables are displayed as the difference between the values obtained immediately after the injection and the correspondent pre-injection condition. Negative values show that the CoP variable decreased after the injection of the saline solution compared to its respective pre-injection condition. Likewise, positive values show that the CoP variable increased after the injection compared to its respective pre-injection condition.

### 2.6. Statistical analysis

Pain outcomes were compared between injection types (isotonic or hypertonic injections) with paired T-tests when normal distribution was present (VAS scores and pain area data) and with the Wilcoxon Signed Rank Test when the data distribution was non-normal (McGill scores). The task measures (number of answers, number of correct answers) were evaluated with a 3-way RM-ANOVA with *injection* (isotonic vs hypertonic), *time* (pre-injection vs after injection) and *task* (counting forward vs backwards) as main factors. The CoP parameters were compared with a 2-Way RM-ANOVA with *task* and *injection* as main factors, and the p-values are shown in the table 3. Bonferroni post-hoc correction for multiple comparisons was applied and p-values are shown in the results texts. The alfa-value ( $\alpha$ ) for statistical significance was set to 0.05.

## 3. Results

### 3.1 Area and amplitude of perceived pain'

Fig. 4 shows the reported pain areas following both isotonic and hypertonic injections. Pain was present in the anterior and lateral portions of the thigh after both isotonic and hypertonic injections, being more concentrated in the lower half of the thigh after the isotonic injections. The hypertonic saline injections induced higher pain area (mean area  $\pm$  SD: isotonic =  $518.6 \pm 690.6$  au; hypertonic =  $1659.3 \pm 1574.0$  au;  $P=0.003$ ) and higher VAS scores (mean score  $\pm$  SD: isotonic =  $0.9 \pm 1.1$  cm; hypertonic =  $4.7 \pm 1.7$  cm;  $P<0.001$ )

than isotonic saline injections. Table 1 shows the scores for each class of words from McGill Pain Questionnaire and the pain rating index. Subjects presented a higher total pain rating index and scored higher in all the categories, with the exception of the affective class, after the hypertonic injections ( $P<0.05$ ).

### 3.2 Cognitive task performance

Only for the analysis of the cognitive task performance, one subject was not included due to problems in the answers recording. The total number of answers and the number of correct answers decreased during backwards counting conditions compared with forwards counting despite the injection effect (significant main effect for *task factor*; Table 2).

### 3.3 Center of pressure

#### Effect of experimental pain in CoP variables

There were no statistical differences between the different conditions for the factor *injection* on any of the CoP variables (Table 3).

#### Effect of cognitive task in CoP variables

A main effect of *task* was found for the CoP AP-velocity ( $F=5.82$ ;  $P=0.028$ ), showing that there was an increased AP-velocity during the counting backwards task compared to the counting forwards task, regardless the type of injection (Table 3).

#### Effect of the interaction between experimental pain and cognitive task in CoP variables

An interaction effect was found between *injection* and *task* factors for CoP total area and CoP ML-velocity (CoP total  $F=7.78$ ,  $P=0.049$ ; CoP ML  $F=4.69$ ,  $P=0.021$ ) (Table 3). Post-hoc comparisons showed that both variables decreased after the hypertonic injection in comparison to the condition with isotonic injection when subjects were counting forward (Bonferroni:  $P = 0.010$  for total area;  $P = 0.015$  for ML-velocity). After the hypertonic injection, CoP total area increased when subjects were counting backwards in comparison to when they were counting forwards (Bonferroni:  $P = 0.019$ ). ML-velocity showed differences between the

different cognitive tasks also after the injection of hypertonic solution, with a smaller decrease of ML-velocity while counting backwards (Bonferroni:  $P = 0.049$ ).

#### 4. Discussion

The present study aimed at quantifying how postural stability, represented by CoP sway (velocity and area of displacement) and CoP complexity (CoP SaEn), is modified during experimental pain while performing a cognitive task. The main results showed that the kind of cognitive task did not interfere with postural stability in the absence of pain. Experimental pain around the knee joint reduced CoP sway but did not affect CoP complexity during the performance of an easier cognitive task. During experimentally induced pain, the performance of a difficult cognitive task increased CoP sway but did not change CoP complexity.

##### Pain intensity and counting performance

The subjects showed higher pain intensity for the hypertonic saline injection and a larger pain area compared with the isotonic saline injection, as expected, indicating that experimental pain occurred (Hirata et al. 2011). The McGill pain questionnaire indicated that hypertonic saline was perceived more impairing than the isotonic injection in all subscales except for the affective one. It is important to note that during isotonic injections subjects rated pain around 1/10, which cannot be classified as a totally pain free condition.

Counting performance requires the use of cognitive process which relies on the working memory of the subject (Lemaire 1996), impairing motor output performance when executed simultaneously with a motor task (Vuillerme and Nafati 2007). Seminowicz and Davis (2007) showed that subjects are able to maintain performance of difficult cognitive task while experiencing different levels of pain. In this study, the painful condition did not affect the counting performance while performing a motor task (standing still) indicating that healthy subjects are able to engage multiple tasks (motor and cognitive) during pain without compromising performance. This suggests that sufficient cognitive resources were available to manage the cognitive process of counting forwards or backwards despite the interpretation of painful stimuli and the postural control task (Eccleston et al. 1999). Finally, education level is associate with both motor and

perceptual performance, where higher education level is associated with better performance (Voos et al. 2015). Since our subjects were all university students, we believe that bias due to education level did not affect the present results.

#### Effect of cognitive tasks on postural stability

Our first initial hypothesis, that (i) the kind of cognitive task (more or less demanding) in a non-painful condition would not interfere with CoP sway or CoP complexity, was confirmed. The factor task affected the CoP anterior-posterior velocity, indicating an increased velocity during the execution of the more difficult task (counting backwards) in comparison to the easier task (counting forward). Nevertheless, the CoP SaEn was not affected by the kind of the performed cognitive task. These results indicate that enough cognitive resources were available to overcome the demands of both cognitive and postural tasks, which was expected since they were young individuals without any sensory-motor alterations.

#### Effect of experimental knee-related pain on postural stability

Our second initial hypothesis, that (ii) experimental pain would increase CoP sway and decrease CoP complexity was not confirmed since the type of saline solution injected did not affect the CoP variables. However, even though the factor *injection* did not show statistical differences between the different conditions for any of the studied CoP variables, there was a difference between total area and ML-velocity between the control and the painful condition when the subjects were counting forwards, i.e., in conditions where the kind of cognitive task performed was the same. Interestingly, during the counting forward, the type of injection resulted significant changes in postural sway (total area and ML-velocity) in opposite directions: positive values of the difference between pre-injection and after injection of the isotonic solution, whereas after the injection of the hypertonic solution both variables showed negative values. Additionally, no significant changes were observed in the structural variability of the CoP signal. This is contrary to the initial hypothesis, where an increase in postural sway and a decrease in structural variability during painful conditions were expected. It is also in contrast with previous findings (Mazaheri et al. 2013) but may relate

to the different position of the feet used in this study, which affects the postural sway (Day et al. 1993). The tandem feet position adopted allows less displacement of the CoP due to the limited base of support compared to side-by-side feet position, since if the subjects increase the CoP amplitude they may fall (Day et al. 1993). This also may reflect a voluntary strategy, requiring a greater amount of cognitive resources and attention (Morasso and Sanguineti 2002), attempting to avoid large excursions of the body and consequent loss of balance. For the current study, this might indicate that the subjects prioritized the balance task over the other tasks, also known as *posture first strategy* (Vuillerme and Nafati 2007). The subjects were able to reduce the postural sway without compromising the counting performance during the easy cognitive task, suggesting that the available cognitive resource was sufficient to perform the less challenging cognitive task without compromising postural stability. Therefore, these results indicate that healthy subjects have the capacity to perform easy cognitive tasks while ensuring postural stability (Siu and Woollacott 2007). Reducing postural sway might reflect a motor strategy available for healthy subjects to avoid excessive translation of the body, which could lead to balance loss (Winter 1995). This strategy was also observed during the control injection while counting backwards, probably indicating that a high cognitive load seems to be interpreted as a threat to postural stability. An alternative explanation for the contrast between the present study and the previous studies with pain patients showing larger postural sway (Schulte et al. 2004; Levinger et al. 2016) might be the pain model used that is not a complete proxy to the impaired pain patients' sensory-motor system.

#### Interactions between pain and cognitive load on postural stability

Our initial third hypothesis, that (iii) the presence of experimental pain would increase CoP sway and decrease CoP complexity only when performing a difficult cognitive task was partially confirmed since CoP sway increased during pain under a difficult cognitive task, but the CoP complexity did not change. ANOVA results showed an interaction between the task and injection factors for total area and ML-velocity. After the hypertonic injection CoP total area increased and CoP ML-velocity decreased less while counting

backwards in comparison to counting forwards condition, corroborating our hypothesis. ANOVA results also showed an effect of the task factor on AP-velocity with post-hoc comparisons showing a difference only during the hypertonic injection condition: while counting backwards AP-velocity also increased. Altogether these results show that CoP sway increases when performing a more demanding cognitive task in the presence of experimental pain. This might reflect an interference with the information-processing capacity and an attention disruption from both postural control and cognitive task (Eccleston et al. 1999). Previous studies suggest that disruptions of sensory information lead to worsening of proprioception in the affected area (Matre et al. 2002), further impairing postural sway (Hirata et al. 2010, 2011). The results indicate that the posture first strategy (Vuillerme and Nafati 2007) found during the easy cognitive task during pain is no longer feasible when a difficult cognitive task is performed during painful conditions. The increased cognitive load in painful conditions seems to impair the motor performance maybe due to insufficient cognitive resource to simultaneously maintain postural stability (which requires significant amount of attention (Morasso and Sanguineti 2002)) and execute a difficult cognitive task. These results might have important new implications in understanding the mechanisms related to fall accidents. Postural stability in daily life activities is usually performed in combination with additional tasks, for example, walking in a busy slippery sidewalk. These daily life activities involves simultaneously competition for the cognitive resources available (Woollacott and Shumway-Cook 2002) to evaluate the environment constrains in order to promote the best motor strategy (Winter 1995). Our present results indicate that, if the subject performs a challenging postural task in pain, his/her capacity for maintain balance while exposed to a difficult cognitive task is suboptimal, which could increase the likelihood of losing balance.

The complexity of postural sway did not show any differences between the experimental conditions. This result is contrary to the literature finding that young healthy subjects present a more regular and less automatic postural sway (decreased CoP SaEn) when the motor task is more difficult (e. g. standing with eyes closed) and more irregular postural sway and more automatic postural sway (increased CoP SaEn) when a



cognitive task is added (Donker et al. 2007; Stins et al. 2009). The fact that the cognitive task did not interfere with CoP complexity may be due to the nature of both motor (standing in tandem position) and cognitive (subtraction calculus) tasks used in the experimental setup that did not interfere with the automaticity of postural control. Besides that, pain also did not affect CoP complexity, showing that experimental knee-related pain did not compromise the coupling between the components of the system responsible for balance in the current experimental setup. Future studies should investigate the interaction between pain, cognition and on CoP complexity with different motor and cognitive demands, in addition to different populations.

Despite interesting results regarding the effects of cognitive tasks in postural control during pain, the relevance of the findings for clinical populations should be interpreted with care. The experimental pain model used here is convenient to assess the effect of pain without the interference of potential structural or pathologies. However, extrapolating the current findings to an older population can only be done to some degree. Additionally, chronic pain patients may also suffer from depressive symptoms (Bair et al. 2003) or anxiety (McWilliams et al. 2003), which might increase cognitive load (Nebes et al. 2001). Furthermore, cognitive impairments are often found in chronic pain patients, decreasing the possibility to maintain performance of two or more concurrent tasks (Brauer et al. 2004), as opposed to what was observed in this study where young healthy subjects were recruited. Also, there was no recording of postural sway without any cognitive task. This would have allowed comparisons with a condition where neither pain nor cognitive tasks were influencing postural sway, and could have reduced type 2 errors given that multiple CoP variables were analyzed in the study. Thus, it can be considered a limitation to our interpretations.

## 5. Conclusions

Pain and cognitive task interfered on postural stability, changing its patterns. During the performance of a simple cognitive task, pain reduced postural sway, while during the performance of a more demanding cognitive task, postural sway was increased in young healthy subjects. Since our subjects were young healthy

subjects, the direct translation of the present results to patients suffering from pain should be done with caution. However, these results may suggest that rehabilitation approaches should take into account that pain not only affects directly the motor system, but may occupy cognitive resources, potentially resulting in poorer performance when performing rehabilitation exercises. Additionally, rehabilitation strategies using both motor and cognitive resources need further investigation to outline the effect of interaction between pain and cognition on the performance during activities of daily life in patients.

#### Compliance with ethical standards

**Funding:** Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121). The authors thank the State of São Paulo Research Foundation (FAPESP) for the Suda Scholarship (FAPESP 2013/06123-7, 2015/00214-6).

**Conflict of Interest:** The authors declare that they have no conflict of interest.

#### REFERENCES

- Andersson G, Hagman J, Talianzadeh R, et al (2002) Effect of cognitive load on postural control. *Brain Res Bull* 58:135–9
- Bair MJ, Robinson RL, Katon W, Kroenke K (2003) Depression and Pain Comorbidity. *Arch Intern Med* 163:2433. doi: 10.1001/archinte.163.20.2433
- Brauer SG, Broome A, Stone C, et al (2004) Simplest tasks have greatest dual task interference with balance in brain injured adults. *Hum Mov Sci* 23:489–502. doi: 10.1016/j.humov.2004.08.020
- Bronstein AM, Buckwell D (1997) Automatic control of postural sway by visual motion parallax. *Exp Brain Res* 113:243–248. doi: 10.1007/BF02450322
- Day BYBL, Steiger MJ, Thompson PD, Marsden CD (1993) Human Body Motion When Standing : Implications for. *J Physiol* 469:479–499
- Donker SF, Roerdink M, Greven AJ, Beek PJ (2007) Regularity of center-of-pressure trajectories depends on the amount of attention invested in postural control. *Exp Brain Res* 181:1–11. doi: 10.1007/s00221-007-0905-4
- Duarte M, Sternad D (2008) Complexity of human postural control in young and older adults during prolonged standing. *Exp Brain Res* 191:265–276. doi: 10.1007/s00221-008-1521-7
- Eccleston C, Baeyens F, Helen P, et al (1999) Pain Demands Attention : A Cognitive-Affective Model of the Interruptive Function of Pain. 125:356–366
- Era P, Sainio P, Koskinen S, et al (2006) Postural balance in a random sample of 7,979 subjects aged 30 years and over. *Gerontology* 52:204–213. doi: 10.1159/000093652

- 014 8 Etemadi Y, Salavati M, Arab AM, Ghanavati T (2016) Balance recovery reactions in individuals with  
015 9 recurrent nonspecific low back pain: Effect of attention. *Gait Posture* 44:123–127. doi:  
016 10 10.1016/j.gaitpost.2015.11.017
- 017 11 Falla D, Farina D, Dahl MK, Graven-Nielsen T (2006) Muscle pain induces task-dependent changes in  
018 12 cervical agonist/antagonist activity. *J Appl Physiol* 102:601–609. doi: 10.1152/jappphysiol.00602.2006
- 019 13 Farina D (2003) Effect of Experimental Muscle Pain on Motor Unit Firing Rate and Conduction Velocity. *J*  
020 14 *Neurophysiol* 91:1250–1259. doi: 10.1152/jn.00620.2003
- 021 15  
022 16 Fewster KM, Gallagher KM, Howarth SH, Callaghan JP (2017) Low back pain development differentially  
023 17 influences centre of pressure regularity following prolonged standing. *Gait Posture*. doi:  
024 18 10.1016/j.gaitpost.2017.06.005
- 025 19 Fraizer E V., Mitra S (2008) Methodological and interpretive issues in posture-cognition dual-tasking in  
026 20 upright stance. *Gait Posture* 27:271–279. doi: 10.1016/j.gaitpost.2007.04.002
- 027 21 Graven-Nielsen T, Arendt-Nielsen L, Svensson P, Jensen TS (1997) Quantification of local and referred  
028 22 muscle pain in humans after sequential i.m. injections of hypertonic saline. *Pain* 69:111–7
- 029 23  
030 24 Ha H, Cho K, Lee W (2014) Reliability of the Good Balance System® for Postural Sway Measurement in  
031 25 Poststroke Patients. *J Phys Ther Sci* 26:121–124. doi: 10.1589/jpts.26.121
- 032 26 Harbourne RT, Stergiou N (2003) Nonlinear analysis of the development of sitting postural control. *Dev*  
033 27 *Psychobiol* 42:368–377. doi: 10.1002/dev.10110
- 034 28  
035 29 Hirata RP, Arendt-Nielsen L, Graven-Nielsen T (2010) Experimental calf muscle pain attenuates the postural  
036 30 stability during quiet stance and perturbation. *Clin Biomech* 25:931–937. doi:  
037 31 10.1016/j.clinbiomech.2010.06.001
- 038 32 Hirata RP, Ervilha UF, Arendt-Nielsen L, Graven-Nielsen T (2011) Experimental muscle pain challenges the  
039 33 postural stability during quiet stance and unexpected posture perturbation. *J Pain* 12:911–919. doi:  
040 34 10.1016/j.jpain.2011.02.356
- 041 35 Hirata RP, Jørgensen TS, Rosager S, et al (2013) Altered Visual and Feet Proprioceptive Feedbacks during  
042 36 Quiet Standing Increase Postural Sway in Patients with Severe Knee Osteoarthritis. *PLoS One* 8:1–8.  
043 37 doi: 10.1371/journal.pone.0071253
- 044 38  
045 39 Huxhold O, Li SC, Schmiedek F, Lindenberger U (2006) Dual-tasking postural control: Aging and the effects  
046 40 of cognitive demand in conjunction with focus of attention. *Brain Res Bull* 69:294–305. doi:  
047 41 10.1016/j.brainresbull.2006.01.002
- 048 42 Kahneman D (1973) *Attention and effort*. Prentice-Hall
- 049 43  
050 44 Kapoula Z, Lê TT (2006) Effects of distance and gaze position on postural stability in young and old subjects.  
051 45 *Exp Brain Res* 173:438–445. doi: 10.1007/s00221-006-0382-1
- 052 46  
053 47 Knoop J, Van Der Leeden M, Van Der Esch M, et al (2012) Association of lower muscle strength with self-  
054 48 reported knee instability in osteoarthritis of the knee: Results from the Amsterdam Osteoarthritis  
055 Cohort. *Arthritis Care Res* 64:38–45. doi: 10.1002/acr.20597
- 056 49  
057 50 Kuczyński M, Szymańska M, Bieć E (2011) Dual-task effect on postural control in high-level competitive  
058 51 dancers. *J Sports Sci* 29:539–545. doi: 10.1080/02640414.2010.544046
- 059 52  
060 53 Larivière C, Butler H, Sullivan MJL, Fung J (2013) An exploratory study on the effect of pain interference and  
061 54 attentional interference on neuromuscular responses during rapid arm flexion movements. *Clin J Pain*  
062 55  
063 56  
064 57  
065 58

- 29:265–275. doi: 10.1097/AJP.0b013e318250ed6f
- Lemaire P (1996) The Role of Working Memory Resources in Simple Cognitive Arithmetic. *Eur J Cogn Psychol* 8:73–104. doi: 10.1080/095414496383211
- Levinger P, Nagano H, Downie C, et al (2016) Biomechanical balance response during induced falls under dual task conditions in people with knee osteoarthritis. *Gait Posture* 48:106–112. doi: 10.1016/j.gaitpost.2016.04.031
- Liston MB, Bergmann JH, Keating N, et al (2014) Postural prioritization is differentially altered in healthy older compared to younger adults during visual and auditory coded spatial multitasking. *Gait Posture* 39:198–204. doi: 10.1016/j.gaitpost.2013.07.004
- Madeleine P, Nielsen M, Arendt-Nielsen L (2011) Characterization of postural control deficit in whiplash patients by means of linear and nonlinear analyses - A pilot study. *J Electromyogr Kinesiol* 21:291–297. doi: 10.1016/j.jelekin.2010.05.006
- Manor B, Costa MD, Hu K, et al (2010) Physiological complexity and system adaptability: evidence from postural control dynamics of older adults. *J Appl Physiol* 109:1786–1791. doi: 10.1152/japplphysiol.00390.2010
- Matre D, Arendt-Nielsen L, Knardahl S (2002) Effects of localization and intensity of experimental muscle pain on ankle joint proprioception. *Eur J Pain* 6:245–260. doi: 10.1053/eujp.2002.0332
- Mazaheri M, Coenen P, Parnianpour M, et al (2013) Low back pain and postural sway during quiet standing with and without sensory manipulation: A systematic review. *Gait Posture* 37:12–22. doi: 10.1016/j.gaitpost.2012.06.013
- Mazaheri M, Heidari E, Mostamand J, et al (2014) Competing effects of pain and fear of pain on postural control in low back pain? *Spine (Phila Pa 1976)* 39:E1518–E1523. doi: 10.1097/BRS.0000000000000605
- McWilliams LA, Cox BJ, Enns MW (2003) Mood and anxiety disorders associated with chronic pain: an examination in a nationally representative sample. *Pain* 106:127–33
- Melzack R (1975) The McGill Pain Questionnaire: major properties and scoring methods. *Pain* 1:277–99
- Mense S (1993) Nociception from skeletal muscle in relation to clinical muscle pain. *Pain* 54:241–89
- Morasso PGP, Sanguineti V (2002) Ankle muscle stiffness alone cannot stabilize balance during quiet standing. *J Neurophysiol* 88:2157–2162. doi: 10.1152/jn.00719.2001
- Nebes RD, Butters MA, Houck PR, et al (2001) Dual-task performance in depressed geriatric patients. *Psychiatry Res* 102:139–151. doi: 10.1016/S0165-1781(01)00244-X
- Pellecchia GL (2003) Postural sway increases with attentional demands of concurrent cognitive task. *Gait Posture* 18:29–34. doi: 10.1016/S0966-6362(02)00138-8
- Peterka RJ (2003) Dynamic Regulation of Sensorimotor Integration in Human Postural Control. *J Neurophysiol* 91:410–423. doi: 10.1152/jn.00516.2003
- Richman JS, Moorman JR (2000) Physiological time-series analysis using approximate entropy and sample entropy Physiological time-series analysis using approximate entropy and sample entropy. *Am Physiol Soc*
- Schulte E, Ciubotariu A, Arendt-Nielsen L, et al (2004) Experimental muscle pain increases trapezius muscle

- activity during sustained isometric contractions of arm muscles. *Clin Neurophysiol* 115:1767–1778. doi: 10.1016/j.clinph.2004.03.005
- Scoppa F, Capra R, Gallamini M, Shiffer R (2013) Clinical stabilometry standardization. Basic definitions - Acquisition interval - Sampling frequency. *Gait Posture* 37:290–292. doi: 10.1016/j.gaitpost.2012.07.009
- Seminowicz DA, Davis KD (2007) Interactions of pain intensity and cognitive load: The brain stays on task. *Cereb Cortex* 17:1412–1422. doi: 10.1093/cercor/bhl052
- Sherafat S, Salavati M, Takamjani IE, et al (2014) Effect of dual-tasking on dynamic postural control in individuals with and without nonspecific low back pain. *J Manipulative Physiol Ther* 37:170–179. doi: 10.1016/j.jmpt.2014.02.003
- Shiozawa S, Hirata RP, Graven-Nielsen T (2013) Reorganised anticipatory postural adjustments due to experimental lower extremity muscle pain. *Hum Mov Sci* 32:1239–1252. doi: 10.1016/j.humov.2013.01.009
- Siu KC, Woollacott MH (2007) Attentional demands of postural control: The ability to selectively allocate information-processing resources. *Gait Posture* 25:121–126. doi: 10.1016/j.gaitpost.2006.02.002
- Slifkin AB, Newell KM (1999) Noise, information transmission, and force variability. *J Exp Psychol Hum Percept Perform* 25:837–851. doi: 10.1037//0096-1523.25.3.837
- Søndergaard KHE, Olesen CG, Søndergaard EK, et al (2010) The variability and complexity of sitting postural control are associated with discomfort. *J Biomech* 43:1997–2001. doi: 10.1016/j.jbiomech.2010.03.009
- Stins JF, Michielsen ME, Roerdink M, Beek PJ (2009) Sway regularity reflects attentional involvement in postural control: Effects of expertise, vision and cognition. *Gait Posture* 30:106–109. doi: 10.1016/j.gaitpost.2009.04.001
- Swan L, Otani H, Loubert P V. (2007) Reducing postural sway by manipulating the difficulty levels of a cognitive task and a balance task. *Gait Posture* 26:470–474. doi: 10.1016/j.gaitpost.2006.11.201
- Vaillancourt DE, Newell KM (2002) Changing complexity in human behavior and physiology through aging and disease. *Neurobiol Aging* 23:1–11. doi: 10.1016/S0197-4580(02)00052-0
- Vaillancourt DE, Newell KM (2000) The dynamics of resting and postural tremor in Parkinson's disease. *Clin Neurophysiol* 111:2046–2056. doi: 10.1016/S1388-2457(00)00467-3
- Van Daele U, Hagman F, Truijien S, et al (2010) Decrease in postural sway and trunk stiffness during cognitive dual-task in nonspecific chronic low back pain patients, performance compared to healthy control subjects. *Spine (Phila Pa 1976)* 35:583–589. doi: 10.1097/BRS.0b013e3181b4fe4d
- Van Ryckeghem DM, Van Damme S, Eccleston C, Crombez G (2018) The efficacy of attentional distraction and sensory monitoring in chronic pain patients: A meta-analysis. *Clin Psychol Rev* 59:16–29. doi: 10.1016/j.cpr.2017.10.008
- Veldhuijzen DS, Kenemans JL, De Bruin CM, et al (2006) Pain and attention: Attentional disruption or distraction? *J Pain* 7:11–20. doi: 10.1016/j.jpain.2005.06.003
- Voos MC, Piemonte MEP, Castelli LZ, et al (2015) Association between Educational Status and Dual-Task Performance in Young Adults. *Percept Mot Skills* 120:416–437. doi: 10.2466/22.PMS.120v18x8
- Vuillerme N, Nafati G (2007) How attentional focus on body sway affects postural control during quiet

1  
2  
3  
4  
5  
6  
7  
8 standing. Psychol Res 71:192–200. doi: 10.1007/s00426-005-0018-2  
9  
10 Winter DA (1995) A. B. C. (Anatomy, Biomechanics, Control) of Balance During Standing and Walking.  
11 Waterloo Biomechanics, Ontario  
12  
13 Woollacott M, Shumway-Cook A (2002) Attention and the control of posture and gait: A review of an  
14 emerging area of research. Gait Posture 16:1–14. doi: 10.1016/S0966-6362(01)00156-4  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

### Figure captions

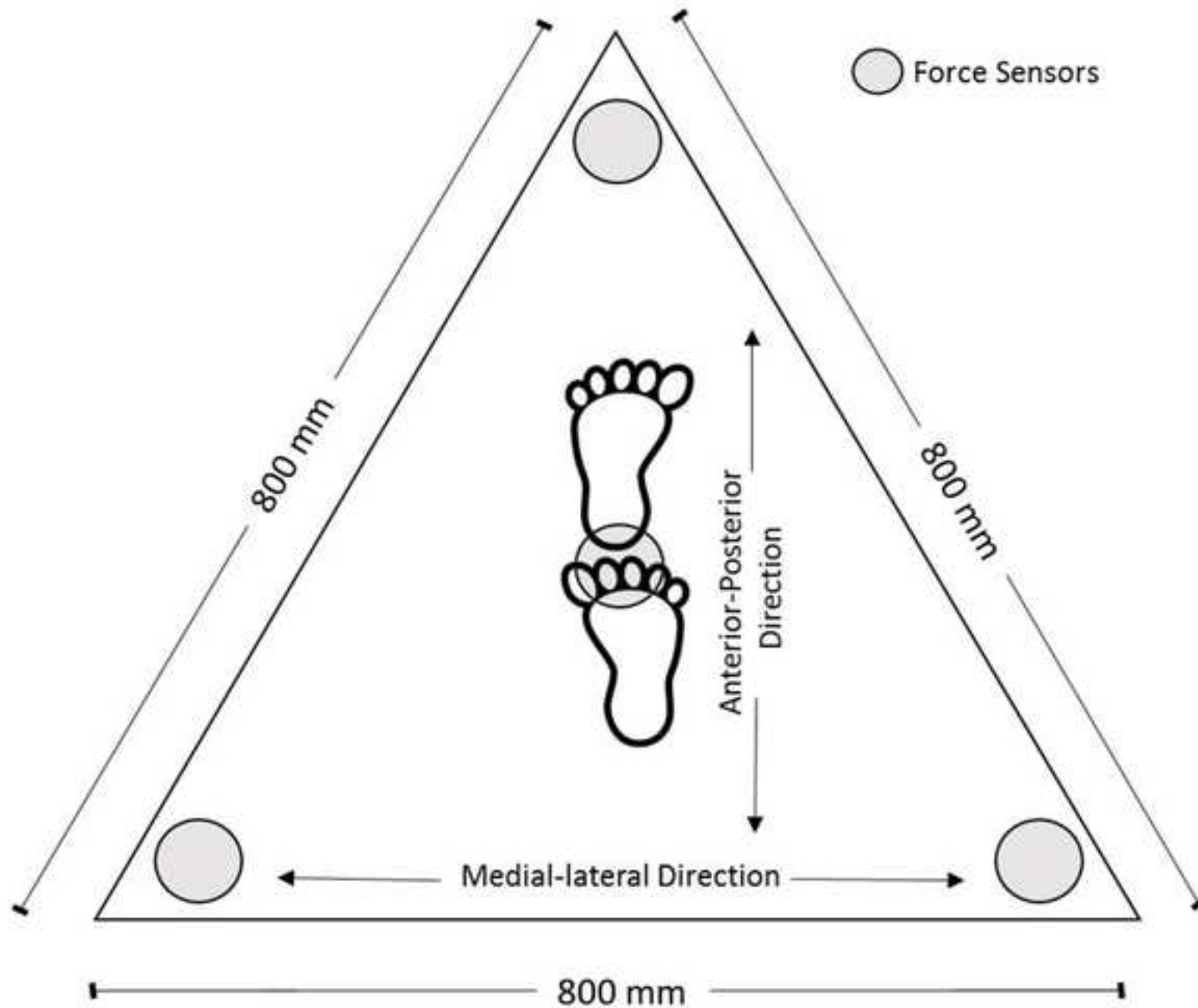
**Fig 1** Schematic drawing representing the force platform size, sensor locations, and the tandem position of the subjects during the experiment

**Fig 2** Study design overview: pain assessments were performed immediately after each injection and each balance measurement; the order of the saline injections was randomized in a balanced way

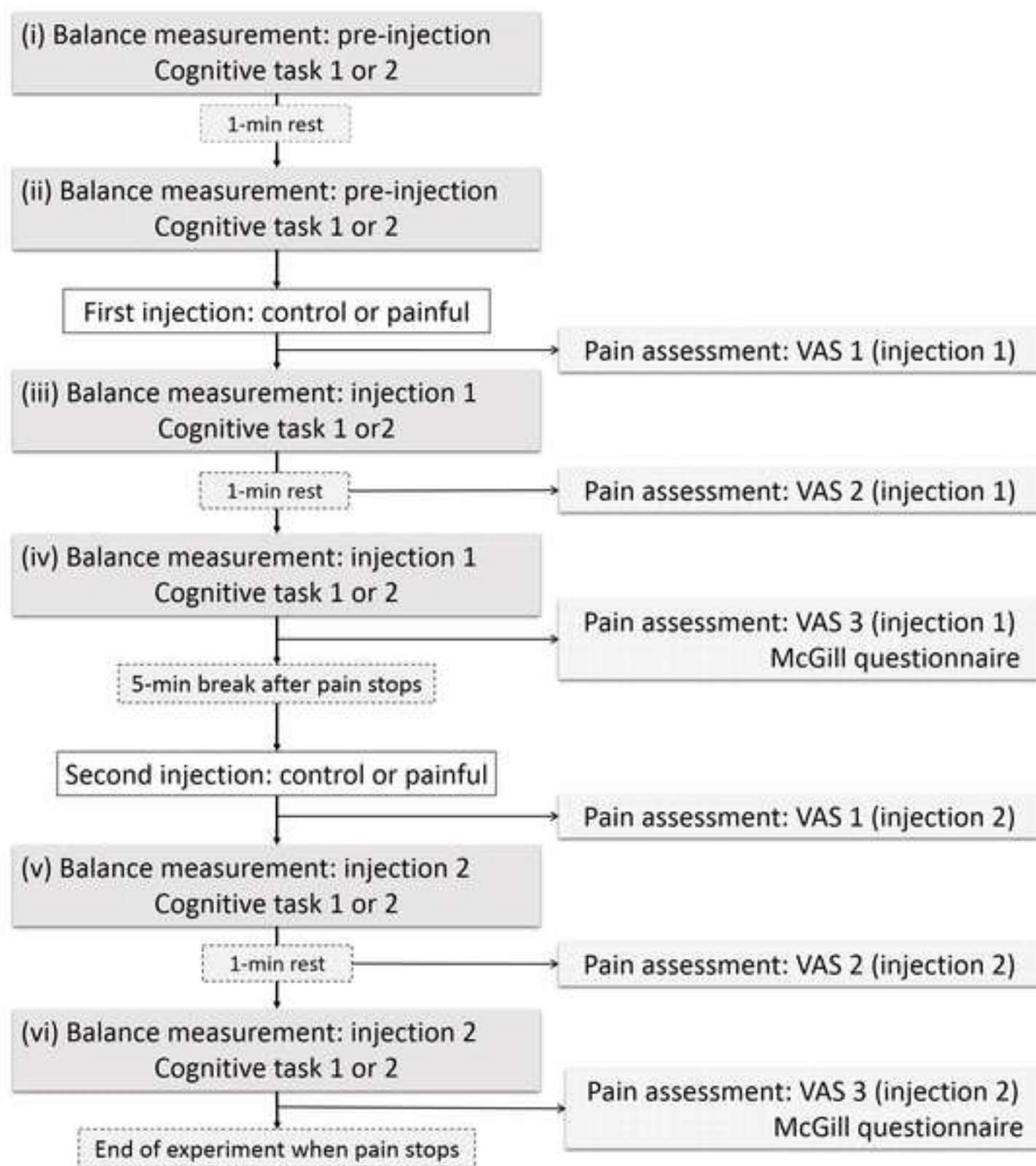
**Fig 3** Injections sites for vastus lateralis muscle, performed at two thirds of the distance from the anterior spina iliaca (a) to the lateral side of the patella (b); and for the vastus medialis muscle, performed 5 cm proximal and 5 cm medial to the medial corner of the patella (c),

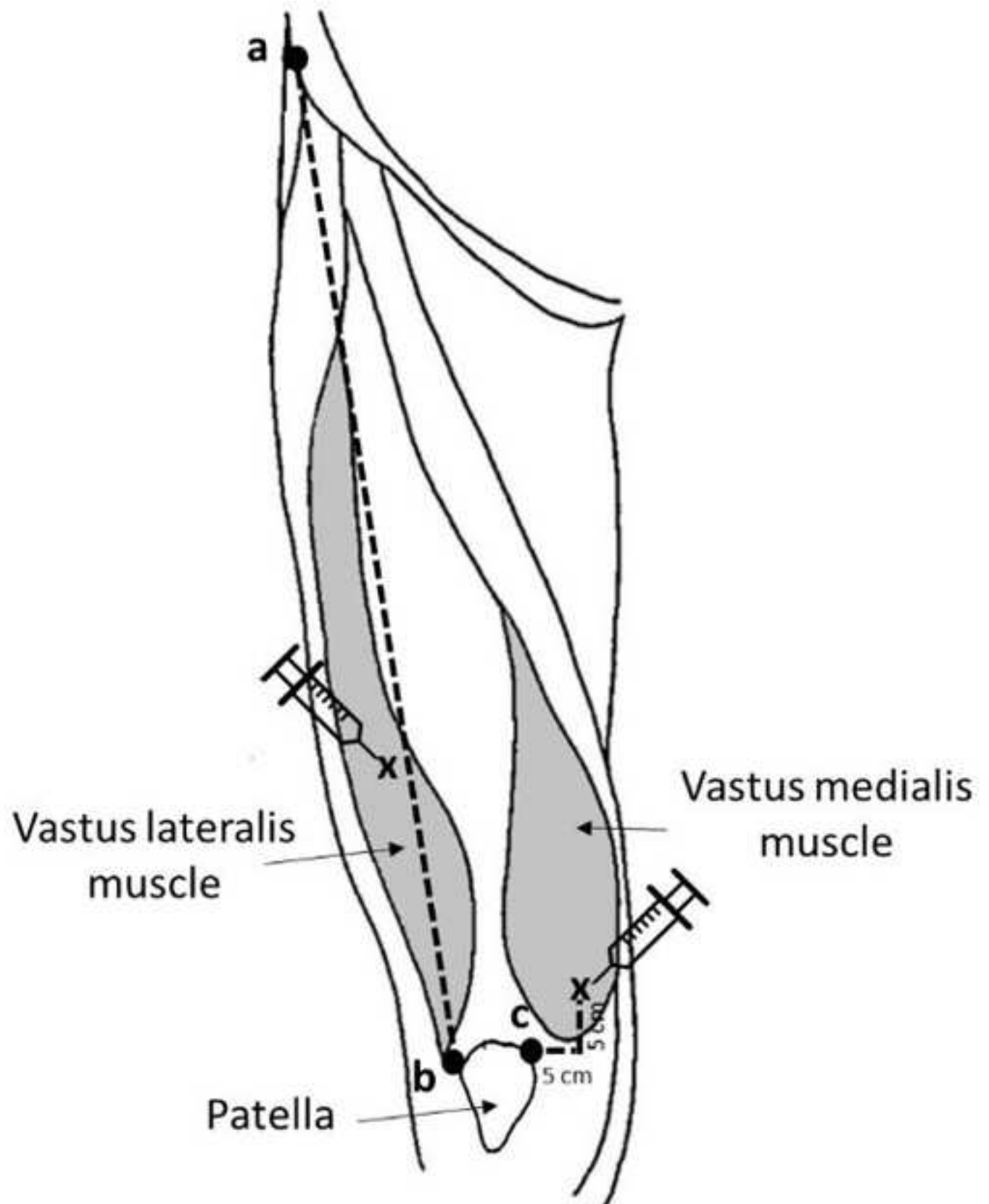
**Fig 4** Representation of the experimental pain distribution reported areas after isotonic (top, blue in the online version) and hypertonic (bottom, red in the online version saline injections (A); the individual distributions are superimposed in the anatomical drawings

Figure 1









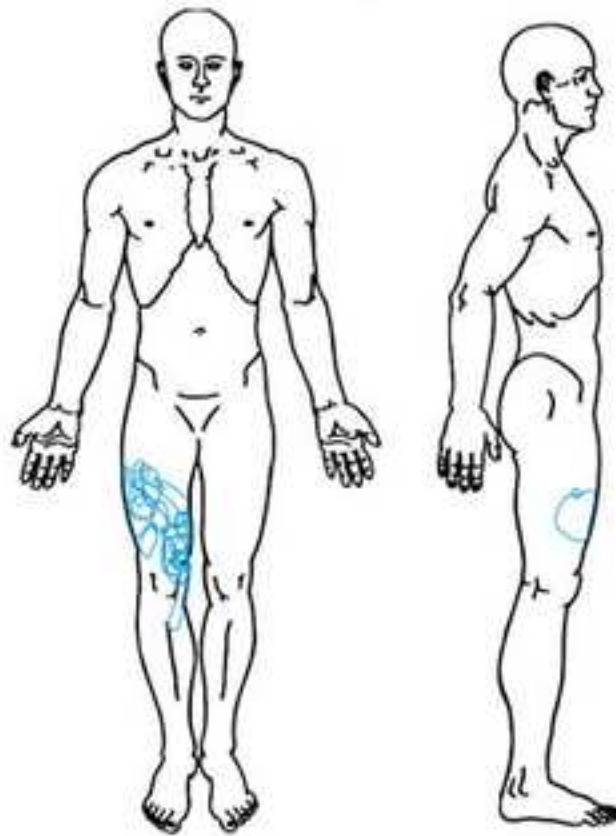
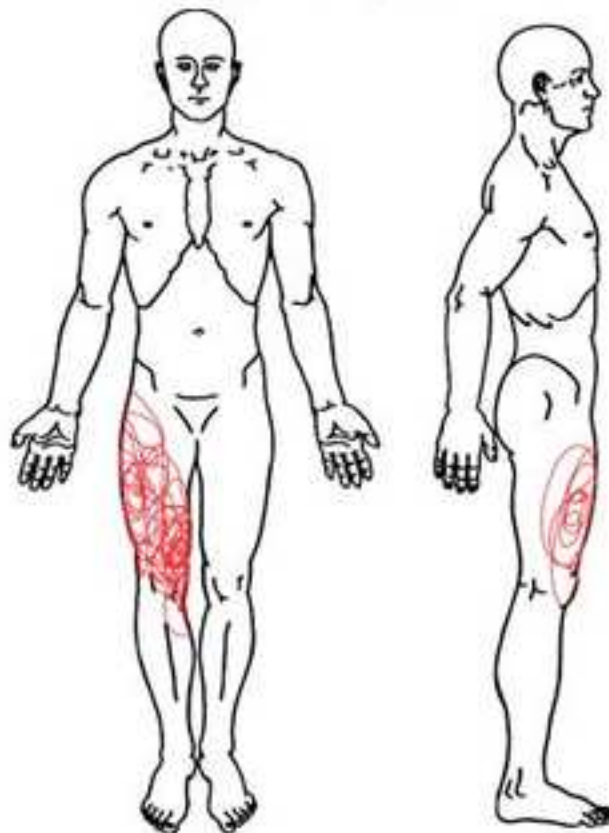
**Isotonic injection****Hypertonic injection**

Table 1 – McGill Pain Questionnaire scores (median [Range]) for each category and total pain rating index for the pain experienced after isotonic and hypertonic injections.

McGill scores	Injection		P-value
	Isotonic	Hypertonic	
Sensory	1 [0-18]	8.5 [2-23]*	0.023
Affective	0 [0-7]	0 [0-4]	0.174
Evaluative	0 [0-1]	1.5 [0-4]*	0.001
Miscellaneous	0 [0-7]	2.5 [0-10]*	0.004
Total pain rating index	2.5 [0-33]	16 [5-30]*	0.001

\*Statistically significant ( $P < 0.05$ ) higher than isotonic condition (Wilcoxon Signed Rank Test with Bonferroni correction).

Table 2 – Mean (±SD) of the cognitive tasks performances before and during both  
injections type (hypertonic and isotonic) and three-way repeated measures ANOVA  
results (F; P).

Formatted: Font: 12 pt

Task performance	Condition	Cognitive task		ANOVA (F; P value)			
		Counting forward	Counting backward	Time	Injection	Task	Time x Injection x Task
Total answers	Before control injection	63.3±7.5	31.3±13.5	0.05; 0.833	0.22; 0.644	<b>68.0; &lt;0.001*</b>	0.28; 0.608
	After control injection	63.5±8.1	30.4±15.0				
	Before painful injection	63.3±10.4	32.1±12.7				
	After painful injection	63.3±9.1	32.3±12.7				
Total correct answers	Before control injection	63.3±7.5	30.9±13.9	0.05; 0.819	0.06; 0.815	<b>64.8; &lt;0.001*</b>	0.39; 0.540
	After control injection	63.5±8.1	29.8±8.1				
	Before painful injection	63.3±10.4	30.9±14.2				
	After painful injection	63.3±9.0	31.3±13.5				

\* Statistically significant (P<0.05).

Table 3 – Mean ( $\pm$ SD) of center of pressure (CoP) variables represented as the difference between the measures after and before each injection (isotonic injection considered as control, hypertonic injection considered as painful) and two-way repeated measures ANOVA results (F; *P*).

CoP Variable	Control injection		Painful injection		ANOVA (F; <i>P</i> value)		
	Counting forward	Counting backward	Counting forward	Counting backward	Injection	Task	Injection x task
Total area (cm <sup>2</sup> )	<b>28.9<math>\pm</math>78.5<sup>a</sup></b>	-25.1 $\pm$ 138.7	- <b>84.5<math>\pm</math>145.5<sup>a, b</sup></b>	<b>12.8<math>\pm</math>53.9<sup>b</sup></b>	1.84; 0.196	0.75; 0.400	<b>7.78; 0.049*</b>
AP Velocity (cm/s)	-0.36 $\pm$ 2.24	-0.07 $\pm$ 1.66	-0.39 $\pm$ 1.82	1.07 $\pm$ 2.35	0.61; 0.446	<b>5.92; 0.028*</b>	1.168; 0.614
ML Velocity (cm/s)	<b>0.98<math>\pm</math>1.93<sup>c, d</sup></b>	<b>-0.73<math>\pm</math>2.23<sup>d</sup></b>	<b>-1.71<math>\pm</math>2.61<sup>c, e</sup></b>	<b>-0.34<math>\pm</math>1.92<sup>e</sup></b>	3.90; 0.067	6.68; 0.697	<b>4.69; 0.021*</b>
AP SaEn (a. u.)	0.007 $\pm$ 0.067	0.003 $\pm$ 0.089	0.041 $\pm$ 0.081	0.001 $\pm$ 0.048	0.73; 0.406	1.51; 0.238	1.01; 0.331
ML SaEn (a. u.)	- 0.019 $\pm$ 0.050	- 0.003 $\pm$ 0.038	- 0.004 $\pm$ 0.045	- 0.104 $\pm$ 0.052	0.12; 0.116	0.12; 0.738	0.10; 0.755

\* Statistically significant ( $P<0.05$ ). <sup>a, b, c, d, e</sup> Statistically significant difference between conditions detected in post-hoc tests ( $P<0.05$ ).

### **Author Contribution Statement**

RPH, TP, NV and TGN conceived and designed research. EYS and TP conducted experiments. EYS and RPH analyzed data. EYS, RPH, ICNS, TP, NV and TGN wrote the manuscript. All authors read and approved the manuscript.